

MONOCLONAL ANTIBODIES

TO

FASCIOLA HEPATICA

by

Mark Douglas GORRELL

A thesis submitted for the degree of
Doctor of Philosophy at the Australian
National University

November 1981



The work recorded in this thesis is my own,
except that specifically acknowledged.

M. Gorrell

M.D. Gorrell

ACKNOWLEDGEMENTS

I am grateful to Dr Mike Howell for his suggestion and excellent supervision of this project.

I thank David Irving and Dr T.J. Higgins for the gel electrophoresis and Wendy Lees for typing and Anne Barton for helping to produce this thesis.

Special thanks go to Anne Barton for the many benefits of her wisdom.

I am grateful for the provision of financial support by the Australian Wool Corporation and facilities and assistance by the Department of Zoology.

ABSTRACT

The aim of this project was the production of hybridomas secreting monoclonal antibodies to antigens of *Fasciola hepatica* in order to facilitate the study of those antigens.

Spleen cell suspensions from rats and sheep infected with *F. hepatica* and mice and rats immunized with fluke antigens were prepared and fused with mouse myeloma cells. Various screening methods, including haemolysis or haemagglutination of fluke antigen-coated sheep red cells and immunoprecipitation in agar, failed to detect hybrid cells secreting anti-fluke antibody. However, one sheep x mouse hybrid cell line was found to secrete sheep immunoglobulin (Ig) of unknown specificity.

An I^{125} protein A radioimmunoassay (RIA) was developed, shown to be more sensitive than the methods listed above, then used for screening hybrid cells for the secretion of antibody to *F. hepatica* antigens. Many of the mouse x mouse but none of the sheep x mouse or rat x mouse hybrid cells secreted anti-*F. hepatica* antibody in quantities detectable by RIA.

A cloned hybridoma, MF1, which resulted from a fusion between P3/NSI-1 Ag4-1 myeloma cells and spleen cells from a CBA mouse immunized with adult fluke soluble somatic antigen-coated syngeneic red cells, was chosen for detailed study. The hybrid nature of MF1 was evidenced by its possession of more chromosomes than its myeloma parent and its secretion of a non-myeloma Ig. The latter

was demonstrated by isoelectric focussing, and polyacrylamide gel electrophoresis followed by fluorography. Unlike most NSI-parented hybridomas, MF1 did not secrete NSI-derived light chains.

By radial immunodiffusion, the monoclonal antibody secreted by MF1 was found to be mouse IgG₁. Therefore, it was purified by *Staphylococcus aureus* protein A sepharose affinity chromatography. An indirect fluorescent antibody method showed that the monoclonal antibody bound to structures associated with adult worm caeca, uterus, testes and tegument. Attempts to isolate the antigen to which the monoclonal MF1 antibody binds were not successful. These attempts involved either immunoprecipitation using *S. aureus* and antigens from C¹⁴leucine-labelled immature worms or protein A sepharose in conjunction with unlabelled antigens. Using RIA, it was found that the antigen to which MF1 antibody binds is shared with a number of other helminths, including *Schistosoma mansoni*, *Mesocostoides corti*, *Nematospiroides dubius*, *Toxocara canis* and *Haemonchus contortus*.

Methods developed in this study could readily be applied to the derivation of further monoclonal antibodies that may be useful for antigenic analysis of *F. hepatica*.

TABLE OF CONTENTS

	Page
Acknowledgements	i
Abstract	ii
Table of Contents	iv
List of Figures	viii
List of Tables	x
List of Abbreviations	xi
Chapter 1 GENERAL INTRODUCTION	1
1.1 Fascioliasis	1
1.1.1 Life cycle	2
1.1.2 Diagnosis and control	4
1.2 Immunology of Fascioliasis	7
1.2.1 Cellular responses	9
1.2.1.1 T cells	11
1.2.1.2 Macrophages	12
1.2.1.3 Eosinophils	15
1.2.1.4 Other cells	18
1.2.2 Antibody Responses	20
1.2.2.1 Detection	20
1.2.2.2 Response to infection	22
1.2.2.3 Function	24
1.2.3 Evasion of Immune Responses	25
1.2.4 Vaccination	31
1.2.5 Antigens of <i>F. hepatica</i>	38
1.3 Hybridoma Technology	46
1.3.1 Theory	46
1.3.2 Cell fusion	49
1.3.3 Screening	50

	Page
1.3.4 Cloning	54
1.3.5 Non-murine hybridomas	55
1.3.6 T cell hybridomas	57
1.3.7 Applications	58
1.3.8 Applications to parasitology	62
1.4 Research Project	64
 Chapter 2 PRODUCTION OF SOMATIC CELL HYBRIDS	65
2.1 Introduction	65
2.2 Materials and Methods	68
2.2.1 Antigens	68
2.2.2 Immunizations and infections	68
2.2.3 Coupling antigens to red cells	71
2.2.4 Cell fusion	72
2.2.5 Cloning in agar	75
2.2.6 Screening clones grown in agar	76
2.2.7 Other antibody assays	77
2.2.8 Cryopreservation of cell lines	79
2.2.9 Karyotype analysis of hybrid cells	79
2.3 Results	80
2.3.1 Sheep x mouse hybrid cells	80
2.3.2 Rat x mouse hybrid cells	83
2.3.3 Mouse x mouse hybrid cells	84
2.4 Discussion	85

	Page
Chapter 3 RADIOIMMUNOASSAYS FOR DETECTING HETEROGENEOUS AND MONOCLONAL ANTIBODIES TO <i>FASCIOLA HEPATICA</i>	90
3.1 Introduction	90
3.2 Materials and Methods	98
3.2.1 Antigens	98
3.2.2 Antibodies	98
3.2.3 Iodination	102
3.2.4 Immunoassay	103
3.3 Results	105
3.4 Discussion	119
Chapter 4 CHARACTERIZATION OF HYBRIDOMAS AND MONOCLONAL ANTIBODIES	124
4.1 Introduction	124
4.2 Materials and Methods	126
4.2.1 Cloning by limit dilution	126
4.2.2 Radioimmunoassay	127
4.2.3 Harvesting monoclonal antibodies	128
4.2.4 Radial immunodiffusion	128
4.2.5 Isoelectric focussing	129
4.2.6 Polyacrylamide gel electrophoresis of secreted cell products	129
4.2.7 Antigen localisation	130
4.2.8 Antigen isolation	132
4.3 Results	134
4.4 Discussion	147

Chapter 5 GENERAL DISCUSSION

Page

154

Bibliography

161

LIST OF FIGURES

	Page
1.1 Life cycle of <i>Fasciola hepatica</i>	3
2.1 Flow chart of the procedure used to produce hybridomas	73
2.2 Diagram of an immunodiffusion test in which rabbit IgG anti-sheep IgG was reacted against the supernatant of a sheep x mouse hybrid cell culture	82
3.1 The direct, competitive RIA	94
3.2 The single indirect RIA	94
3.3 The protein A single indirect RIA	95
3.4 The double indirect RIA	95
3.5 Diagram of events in a solid phase single indirect RIA using I ¹²⁵ RAM and soft PVC trays to detect mouse antibodies to <i>F. hepatica</i> antigens	99
3.6 Diagram of the I ¹²⁵ protein A solid phase double indirect RIA used to detect antibodies to <i>F. hepatica</i> antigens	100
3.7 Flow chart of the procedure employed to detect antibodies to <i>F. hepatica</i> antigens by solid phase RIA	104
3.8 Single indirect RIA. Titrations to determine optimum concentrations of <i>F. hepatica</i> antigen FHS and I ¹²⁵ RAM	110
3.9 Double indirect RIA. Titrations to determine optimum concentrations of RAM and I ¹²⁵ protein A	111
3.10 Determinations of optimum times of incubation of PVC trays with <i>F. hepatica</i> antigen FHS, mouse antiserum, secondary antibody and I ¹²⁵ protein A	112
3.11 Determination of the titre of a mouse antiserum to the fluke antigen preparation FHS by I ¹²⁵ protein A solid phase double indirect RIA	113

3.12	An example of applying the I^{125} RAM single indirect RIA to screening hybrid cell supernatants	117
4.1	Isoelectric focussing in 10% polyacrylamide of X63 and MF1 antibodies	140
4.2	Fluorographs of PAGE of C^{14} leucine-labelled secreted products of NSI, X63 and MF1 cells	141
4.3	Antigen localisation. Binding by MF1 antibody to the basal membrane of the tegument shown by a fluorescent antibody method.	142
4.4	Antigen localisation. Specific fluorescence due to MF1 antibody binding to testes tubules and collecting ducts	143
4.5	Antigen localisation. Specific fluorescence due to MF1 antibody binding to caecal cells and testes tubules	144
4.6	Attempted antigen isolation. SDS-PAGE of the pH 3 eluate from protein A sepharose after incubation at pH 8 with MF1 supernatant then FHS	145
4.7	Attempted antigen isolation. Fluorograph of SDS-PAGE of soluble somatic components of <i>F. hepatica</i> biosynthetically labelled with C^{14} leucine and those eluted from Staph A preincubated with X63 IgG ₁ and MF1 IgG ₁	146

LIST OF TABLES

	Page
3.1 Control incubations used in radioimmunoassays	114
3.2 Titres of mouse sera tested by radioimmunoassay and indirect haemagglutination	115
3.3 Titres of sera tested by radioimmunoassay and indirect haemagglutination	116
3.4 Yields of hybrid cells from mouse x mouse fusions, numbers secreting antibody and their fate	118
4.1 Species specificity test of monoclonal antibody MF1 by radioimmunoassay	139

LIST OF ABBREVIATIONS

ADCC	Antibody dependent cell mediated cytotoxicity
AFC	Antibody forming cell
BCG	Bacille Calmette-Guérin
BSA	Bovine serum albumin
C1q, C3, C5a	Components of complement
CBS	Citrate buffered saline. pH 3, 0.1 M citrate, 0.15 M NaCl
cpm	Counts per minute
DASS	Defined antigen substrate spheres
DMM	Dulbecco's modified Eagles medium
DMS	Dimethyl suberimidate 2 HCl
DTH	Delayed type hypersensitivity
EBV	Epstein-Barr Virus
ECF-A	Eosinophil chemotactic factor of anaphylaxis
ELISA	Enzyme linked immunosorbent assay
F ₁	First generation
Fab	Antigen binding fragment of immunoglobulin
Fc	Complement binding fragment of immunoglobulin
FCA	Freund's complete adjuvant
FHH	Adult <i>F. hepatica</i> somatic antigen
FHS	Adult <i>F. hepatica</i> soluble somatic antigen
FiCA	Freund's incomplete adjuvant
FIP	Adult <i>F. hepatica</i> incubation products
GLDH	Glutamate dehydrogenase
H2	Murine transplantation antigen complex
HAT	Hypoxanthine, aminopterin and thymidine

HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HGPRT	Hypoxanthine guanine phosphoribosyl transferase
HLA	Human transplantation antigen complex
ID	Immunodiffusion
IEF	Isoelectric focussing
IEP	Immuno-electrophoresis
Ig	Immunoglobulin
IHA	Indirect haemagglutination
IR	Serum from an infected rat
K cells	Killer cells
MA	Mouse antiserum
MBP	Major basic protein
MCA	Monoclonal antibodies
MF	Mouse anti- <i>F. hepatica</i> hybridoma
MFIP	Incubation products of newly excysted <i>F. hepatica</i> metacercariae
MRBC	Mouse red blood cells
NK cells	Natural killer cells
NSF	1% FCS in 0.15 M NaCl
NSI	P3/NSI-1 Ag4-1 mouse myeloma
PA	Protein A
PAGE	Polyacrylamide gel electrophoresis
PB	Phosphate buffer. pH 7.6, 0.1 M phosphate
PBS	Phosphate buffered saline. pH 7.4, 0.02 M phosphate, 0.15 M NaCl
P'BS	Phosphate buffered saline. pH 7.0, 0.02 M phosphate, 0.15 M NaCl

PEG	Polyethylene glycol
PFC	Plaque forming cells
PVC	Polyvinyl chloride
RA	Rat antiserum
RAM	Rabbit IgG anti-mouse IgG
RAR	Rabbit IgG anti-rat IgG
RAS	Rabbit IgG anti-sheep IgG
RBC	Red blood cell
RIA	Radioimmunoassay
RPMI	Defined culture medium RPMI 1640
SDS	Sodium dodecyl sulphate
SRBC	Sheep red blood cell
Staph A	Heat-killed, fixed <i>Staphylococcus aureus</i> organisms
T ₀ , T ₁ , T ₂	Types of ^{granules from} <i>F. hepatica</i> tegumental cells
T _c	Cytotoxic T cell
TCA	Trichloroacetic acid
TCGF	T cell growth factor
X63	P3/X63 Ag8-1 mouse myeloma

Chapter 1

GENERAL INTRODUCTION

Parasites are a major cause of disease in people of developing countries and domestic animals throughout the world (WHO 1979). The use of chemicals has been found to be inadequate for the long term control of these organisms. This is particularly true of malaria (Bloom 1979) and the parasitic nematodes of sheep (Prichard, Hall, Kelly, Martin and Donald 1980).

Although *Fasciola hepatica*, the sheep liver fluke, has not yet exhibited anthelmintic resistance this event must be anticipated and alternatives such as immunological control investigated. Such investigations are necessarily being preceded by studies of host immune responses and the mechanisms whereby parasites avoid them.

A problem frequently referred to by parasite immunologists is the complexity of their material. As many as 50 *F. hepatica* molecules have been found to be antigenic (Hillyer and Cervoni 1978). Despite this problem, vaccines against many parasites, notably *Plasmodium falciparum* and *Schistosoma mansoni*, may become available in the foreseeable future (Mitchell 1979b; Kagan 1980). This optimism can be attributed mainly to the advent of new technologies: - most notably hybridomas and recombinant DNA. The former provides a means of obtaining large amounts of antibody with a single, predetermined specificity (monoclonal antibody) that can be used to study its corresponding antigen. The latter should enable the mass production of individual antigens by inserting the corresponding DNA sequence into a bacterium.

1.1 FASCIOLIASIS

1.1.1 Life Cycle

The common liver fluke, *Fasciola hepatica* Linnaeus, 1758, is a monozoic trematode which parasitises a wide range of hosts. Its definitive hosts include man, sheep, cattle, horses, rabbits, goats and several marsupials. Infection of humans is a public health problem in some countries (Wattre, Capron and Capron 1978; WHO 1979) and usually follows the eating of watercress (*Nasturtium officinale*). Infections of sheep and cattle are widespread in most countries but fascioliasis is a particular problem in sheep because the worms can survive for many years in this host (Leiper 1938; Durbin 1952). The estimated annual cost to the Australian meat and wool industries is \$20 million (Barger, Dash and Southcott 1978).

The prevalence of fascioliasis varies with rainfall because the parasite is obliged to spend part of its life cycle in water and an amphibious snail (Fig. 1) and miracidia, cercariae and metacercariae are vulnerable to dessication (Boray and Enigh 1964; Meek and Morris 1979a). Hermaphroditic adult flukes reside in the common bile duct of the definitive host. Eggs released by the adult are passed in the faeces. After embryonation, which requires 9 to 14 d at 26°C, the free-swimming miracidium hatches. Upon entering a lymnaeid snail, which in Australia at present is almost always *Lymnaea tomentosa* but could be *L. columella* or *L. viridis* (Boray 1978), it sheds its ciliated epithelium and becomes a sporocyst. Numerous rediae then cercariae are produced inside the intermediate host snail from a single miracidium. Cercariae, which use a tail for swimming, leave the snail and encyst on foliage to

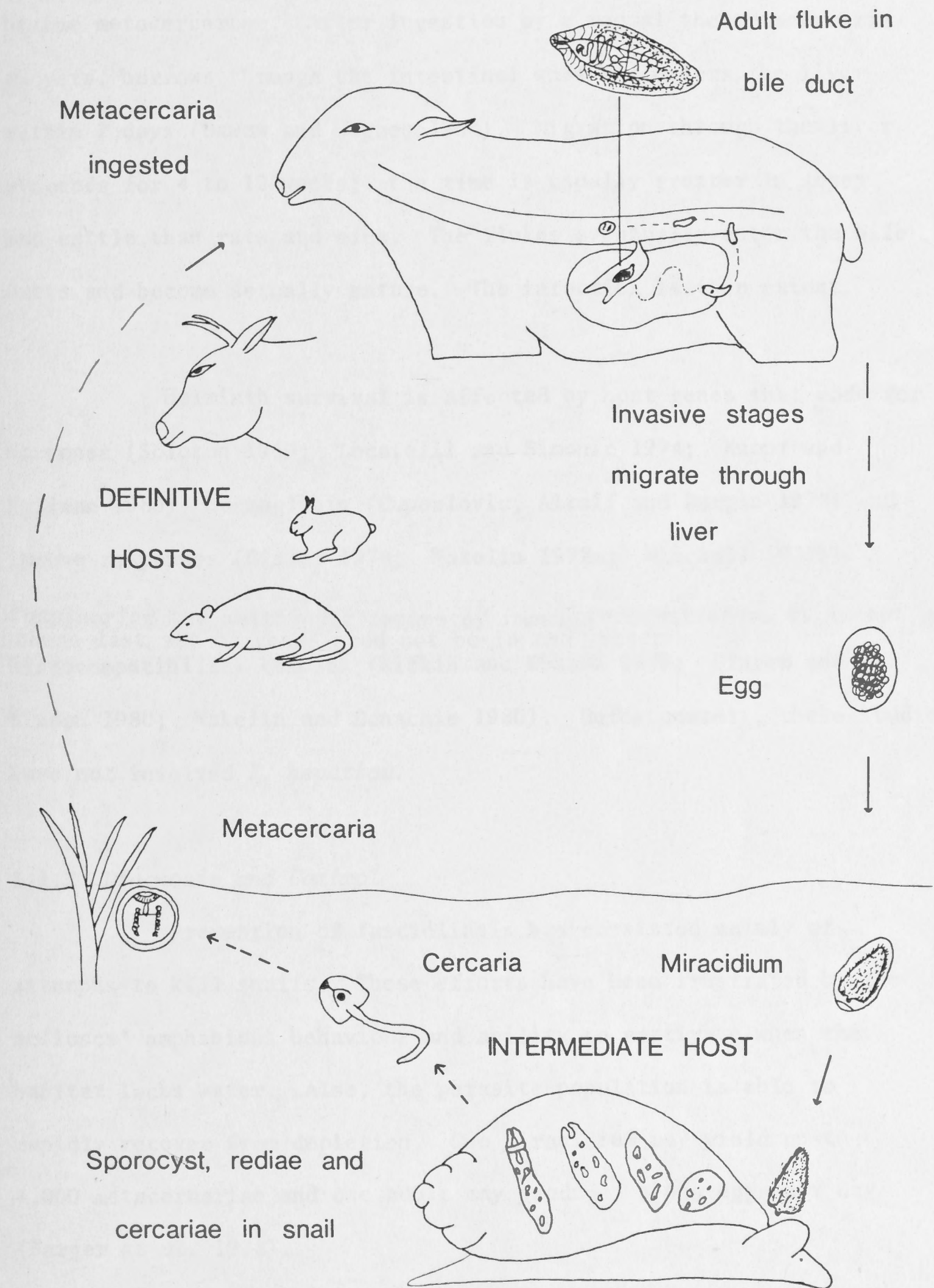


Fig. 1.1 LIFE CYCLE OF *FASCIOLA HEPATICA*

become metacercariae. After ingestion by a mammal the metacercaria excysts, burrows through the intestinal wall and enters the liver within 2 days (Dawes and Hughes 1964). Migration through the liver proceeds for 4 to 12 weeks; the time is usually greater in sheep and cattle than rats and mice. The flukes eventually enter the bile ducts and become sexually mature. The infection is then patent.

Helminth survival is affected by host genes that code for hormones (Solomon 1969; Locatelli and Simoncic 1974; Knopf and Soliman 1980), haemoglobin (Cuperlovic, Altaif and Dargie 1978) and immune responses (Gisler 1978; Wakelin 1978a; Mitchell 1979b).

Considering the multigenic nature of immune responsiveness it is not surprising these last set of genes need not be in the major histocompatibility complex (Rifkin and Dobson 1979; Dineen and Windon 1980; Wakelin and Donachie 1980). Unfortunately, these studies have not involved *F. hepatica*.

1.1.2 *Diagnosis and Control*

Prevention of fascioliasis has consisted mainly of attempts to kill snails. These efforts have been frustrated by the molluscs' amphibious behaviour and ability to aestivate when the habitat lacks water. Also, the parasite population is able to rapidly recover from depletion. One miracidium may yield up to 4,000 metacercariae and one adult may produce 2,000 eggs per day (Barger *et al.* 1978).

Symptoms of the disease are usually noticed during the acute phase of infection when flukes have grown but are still migrating through the liver. Apart from direct physical damage, flukes may precipitate infectious necrotic hepatitis (Black disease), caused by *Clostridium oedematiens*, unless the sheep is vaccinated against this bacterium. Various anthelmintic chemicals are available for treating fluke infections of 4 or more weeks duration (Barger *et al.* 1978). However, diagnosis may be difficult at the early stages of infection. Accurate diagnosis can be made after necropsy or discovery of eggs in the faeces if the infection is patent. Immuno-diagnostic kits are now available (Kagan 1979) but, since they are not specific for *F. hepatica* and may detect antibody stimulated by a past infection, the results need to be interpreted in the light of clinical symptoms.

The possibility of detecting active fluke infections by measuring enzymes in serum following their release from damaged liver cells has been investigated with various assays using mice (Bundesen and Janssens 1971), rats (Thorpe 1965c; Campbell and Barry 1970; Haroun, Hammond and Sewell 1980a,b; Rajasekariah and Howell 1980), rabbits (Bolbol, Hammond and Sewell 1978; Haroun *et al.* 1980b), cattle (Ross, Todd and Dow 1966; Doyle 1973a; Knight 1978; Rowlands and Clampitt 1979) and sheep (Thorpe and Ford 1969; Sinclair 1973, 1975; Knight 1978; Rowlands and Clampitt 1979; Said and Handlos 1980; Sandeman and Howell 1980, 1981b; Sykes, Coop and Robinson 1980b). Most commonly, glutamate dehydrogenase (GLDH) has been measured because, although not liver-specific, it is the most easily detected. At best, GLDH is detectable in sheep serum 8 weeks

after a trickle infection has been initiated (3 metacercariae daily, Sykes *et al.* 1980b) or 3 weeks after the administration of 100 metacercariae (Sandeman and Howell 1980). The presence of GLDH in serum does not indicate the cause of its release, but permits discrimination between extinct and active infections. Thus, it has proved useful as a measure of anthelmintic and vaccine efficacy (Haroun *et al.* 1980a; Rajasekariah and Howell 1980).

A new approach that may overcome many problems of immunodiagnosis is to detect circulating parasite antigens. Immunodiagnostic assays for detecting circulating antigens have been developed for toxoplasmosis (Araujo and Remington 1980), taeniasis (Harrison 1978) and schistosomiasis (M. Capron, Carlier, Nzeyimana, Minoprio, Santoro, Sellin and Capron 1980; Carlier, Nzeyimana, Bout and Capron 1980). Compared with the tests discussed above, the number of antigens likely to be involved is reduced, which facilitates development of specific assays, and a positive test result is evidence of a current infection.

Detectable amounts of parasite antigens in serum are a consequence of a wide variety of parasitic infections (Wilson 1978; Kagan 1979; Sogandares-Bernal, Race, Dennis and Voge 1981) including fascioliasis (Ambroise-Thomas, Desgeorges and Bouttaz 1980; Robert, Leynia de la Jarrige, Chabasse, Mahaza, Bizon and Genthon 1980). Ambroise-Thomas *et al.* (1980) developed an enzyme-linked immunosorbent assay (ELISA) for circulating *F. hepatica* antigens that was successful in detecting a high proportion of bovine infections with few false negatives.

1.2 IMMUNOLOGY OF FASCIOLIASIS

A large part of the literature on fascioliasis contains evidence that all hosts - sheep, cattle, pig, rabbit, guinea pig, rat and mouse - respond immunologically to infection. Both cellular and humoral responses occur. However, argument persists regarding the effectiveness of these responses in regulating fluke infection. A proportion of flukes die during almost all infections but the extent to which acquired immune responses are responsible is still debated. The simplest, though not conclusive, test is to measure the survival of a challenge infection. By this measure most hosts of *F. hepatica* successfully resist reinfection. The exceptions are mice and sheep.

Resistance to challenge in rabbits has been detected by Kerr and Petkovich (1935), Ross (1966a), Kendall Herbert, Parfitt and Peirce (1967), Kendall and Sinclair (1971), Fortmeyer (1973) and Bolbol *et al.* (1978) but conflicting results have been obtained by Hughes (1962a), Cuperlovic, Movsesijan, Borojevic and Lalic (1970) and Haroun *et al.* (1980b). This indicates that the circumstances that permit expression of resistance in this host are poorly defined.

Cattle strongly resist reinfection (Ross 1965, 1966b, 1967b; Boray 1967b; Doyle 1971, 1973a; Nansen 1975; Kendall, Sinclair, Everett and Parfitt 1978) and eliminate most of a primary infection after about 5 months (Ross 1967a; Boray 1969; Doyle 1971, 1972). Pigs strongly resist infection (Ross, Dow and Todd 1967; Nansen, Andersen, Harmer and Riising 1972) and guinea pigs kill a challenge

infection (Vernes, Biguet, Floch and Lefebvre 1972) as do rats (Thorpe and Broome 1962; Hayes, Bailer and Mitrovic 1972, 1973, 1974a; Hayes and Mitrovic 1977; Hayes 1978; Goose and Macgregor 1973; Armour and Dargie 1974; Corba and Spaldonova 1975; Hughes, Harness and Doy 1976a, 1977; Rajasekariah and Howell 1977a, 1978b; Doy, Hughes and Harness 1978; Kelly and Campbell 1979; Haroun *et al.* 1980b; Chapman and Mitchell 1981a).

Some work has found that mice resist a challenge infection (Lang 1967, 1968). However, this finding has been contradicted by other experiments (Hughes 1962a,b; Dawes and Hughes 1970; Gold and Lengy 1972; Harness, Hughes and Doy 1976; Harness, Doy and Hughes 1977; Chapman and Mitchell 1981a). Furthermore, Rajasekariah, Mitchell, Chapman and Montague (1979b) and Lehner and Sewell (1979) failed to immunise mice in the manner successfully employed by Lang (1976) and Lang and Hall (1977). Passive transfer of serum from infected to naive mice also failed to induce protection (Dawes and Hughes 1964). Therefore, it appears that the responses by mice against fluke are ineffective.

In sheep, flukes of challenge infections are no less successful in becoming established than those of primary infections (Kendall 1967; Boray 1967a; Sinclair 1971a, 1973, 1975; Rushton 1977; Kelly and Campbell 1979; Meek and Morris 1979b; Knight 1980a) but retardation of their development has been detected (Sinclair 1971a, 1973; Rushton 1977; Campbell, Gregg, Kelly and Dineen 1978a; Knight 1980a).

1.2.1 Cellular Responses

All mammals infected with fluke respond with extensive hepatic infiltration of leukocytes. Another result of fascioliasis in the liver is collagen deposition, which probably impedes the progress of migratory parasites (Murray and Rushton 1975). In cattle, fibrosis of the liver is particularly severe and the bile ducts calcify; as a result, the survival of flukes of both primary and secondary infections is impaired (Ross 1965, 1966a, 1966b, 1967a; Boray 1967b, 1969; Kendall 1967; Kendall *et al.* 1978; Doyle 1971, 1972). In addition to this fibrotic response cattle immunologically attack fluke, evidenced by serum and cell transfers (Corba, Armour, Roberts and Urquhart 1971; Dargie, Armour, Rushton and Murray 1974).

Nansen *et al.* (1972) found that pigs also respond with both liver fibrosis and encapsulation of parasites by leukocytes. Although sheep do not exhibit an acquired immune response that is lethal to fluke, experiments showing that impairment or enhancement of cellular responses alters liver pathology (Sinclair 1970, 1971b; Dargie, Armour and Urquhart 1973) have led to arguments that hepatic fibrosis in all hosts has an immunological basis (Murray and Rushton 1975; Eriksen 1980b; Section 1.2.1.2). If this is so the relative roles of cells in direct attack and via fibrosis will need to be reevaluated.

Experiments by Dodd and O'Nuallain (1969) suggest that the cellular response may be important in rabbits also.

In mice, Lang, Larsh, Weatherly and Goulson (1967) demonstrated passive transfer of resistance with peritoneal cells. Electron microscopy has revealed *in vivo* cytoadhesion to immature flukes (Koie, Nansen and Christensen 1977; Eriksen 1980b). Nude mice may (Eriksen 1980a) or may not (Rajasekariah *et al.* 1979b) die more rapidly than reconstituted mice after infection with flukes. These findings suggest that cellular responses may at least reduce host pathology.

It appears that neither spleen nor thymus is essential for expression of resistance to infection in rats (Hayes, Bailer and Mitrovic 1975; Hughes, Harness and Doy 1979) yet immunity can be passively transferred with lymph node and spleen cells (Corba *et al.* 1971; Armour and Dargie 1974; Dargie *et al.* 1974).

The results listed above do not indicate which cells or whether cells, rather than antibodies, are responsible for resistance (Mitchell 1979b) since the spleen and lymph node cells contain many cell types, including a high proportion of B cells. The leukocytes in the liver during infection have been found to be mainly eosinophils, plus neutrophils, macrophages and lymphoblasts (Murray and Rushton 1975; Rajasekariah and Howell 1981). However, there is little information on the leukocyte subsets that challenge infections encounter in the intestinal wall (Doy *et al.* 1978; Knight 1980b) even though this is thought to be a major site of fluke death in challenged rats (Hayes and Mitrovic 1977; Hayes 1978; Rajasekariah and Howell 1977a, 1980; Campbell, Kelly and Dineen 1978b; Doy *et al.* 1978).

The possible roles of each cell type are discussed below.

1.2.1.1 *T Cells*

Thymus derived cells (T cells) and B cells make up the subset of leukocytes called lymphocytes.

T cells can be cytotoxic independent of antibody and complement. Although most cells influence their neighbours, T cells appear to have a special role in controlling immune responses by acting as suppressor or helper cells (Gupta and Good 1979b). Helper T cells, usually with the aid of macrophages, induce antibody responses to T-dependent antigens, which include membrane bound and most soluble antigens (antibody responses to other antigens occur after macrophages stimulate B cells). Thus, it would be expected that T cells are essential for host responses to *F. hepatica* as is true of most parasites (Wakelin 1978b; Mitchell 1979a; Rose, Ogilvie, Hesketh and Festing 1979). This has been observed by Eriksen (1980a,b) in mice and Flagstad, Andersen and Nielsen (1972) in calves but, surprisingly, not in rats (Hughes *et al.* 1979). The results obtained by Hughes *et al.* (1979) may have been due to the persistence of T cells in congenitally athymic animals (Brooks, Webb, Robins, Robinson, Baldwin and Festing 1980; Ishikawa and Saito 1980) although this is unlikely (Hughes, pers. comm.).

A search for cytotoxicity by T cells against schistosomes has yielded negative results (Butterworth, Vadas, Martz and Sher 1979a). It would nevertheless be valuable to investigate whether T cells have a direct role in immune responses against flukes.

1.2.1.2 Macrophages

The possibility of an effect of macrophages on the survival of a pathogen is often assessed by testing the effectiveness of BCG administration in protecting the host against challenge. The main effect of correctly presented BCG is macrophage activation, although proliferation of granulocytes also occurs. The phagocytic and proliferative abilities of nonactivated macrophages (monocytes) are limited, they cannot perform antibody dependent cell mediated cytotoxicity (ADCC) (Van Zeijst, Stewart and Schlesinger 1978; Theofilopoulos and Dixon 1979).

BCG can protect mice against challenge with various parasites including *Babesia microti*, *B. rhodaini* and *Plasmodium vinckei* (Allison and Clark 1978), *Trichinella spiralis* (Grove and Civil 1978), *Mesocestoides corti* (Thompson and Penhale 1978) and *Schistosoma mansoni* (Bout, Dupas, Carlier, Afchain and Capron 1977a; Civil, Warren and Mahmoud 1978; Tribouley, Tribouley-Duet and Appriou 1979). Similar effects have been observed with *Echinococcus multilocularis* in cotton rats (Rau and Tanner 1975; Reuben, Tanner and Rau 1978) and *S. mansoni* in hamsters (Capron and Lesoni 1969).

A single intraperitoneal injection of BCG appears unable to protect rats against flukes (Thompson and Howell 1979) but a role for macrophages in fascioliasis, proposed by Eriksen (1980a), cannot be excluded. Although intraperitoneal inoculation of BCG has been effective when the target parasite resides in this hostile environment, it is ineffective against migratory schistosomes, which

are killed in the skin, lungs and liver by intravenous injections of BCG (Civil *et al.* 1978). In addition, two injections of BCG are far more effective than one (Capron and Lesoni 1969; Tribouley *et al.* 1979) and supplementation with immune serum may be necessary to kill schistosomes (Maddison, Kagan, Chandler, Gold, Hillyer, Slemenda and Tsang 1979). Perhaps, by varying the protocol, a protective effect against fascioliasis may be observed.

An indirect role of macrophages may be that of recruiting fibroblasts and inducing them to lay down collagen (Allison, Davies and Clark 1977). Allison and Clark (1978) have suggested that this mechanism is responsible for fibrosis in schistosomiasis. It may be that macrophages mediate the fibrosis that retards flukes of a challenge infection and which appears to have an immunological basis (Murray and Rushton 1975; Eriksen 1980a).

Macrophages phagocytose opsonised (coated with antibody) and non-opsonised non-self material and immune complexes. However, metazoan parasites are too large for phagocytosis so other mechanisms of cellular attack must be used, such as ADCC (Walker 1977). Using ADCC, macrophages have been found to bind and damage helminths including *E. multilocularis* (Baron and Tanner 1977), *Nippostrongylus brasiliensis* (Taylor, Mackenzie and Ogilvie 1979) and *S. mansoni* (Capron, Dessaint, Joseph, Rousseaux, Capron and Bazin 1977; Joseph, Capron, Butterworth, Sturrock and Houba 1978; Kassis, Aikawa and Mahmoud 1979a; Mahmoud, Peters, Civil and Remington 1979). In the rat, baboon and man, cytophilic IgE is involved in this ADCC (Capron,

Dessaint, Capron and Bazin 1975b; Capron *et al.* 1977; Joseph, Dessaint and Capron 1977; Joseph *et al.* 1978) while in the mouse it is opsonic IgG_{2a} (Kassis *et al.* 1979a).

Another 2 immune mechanisms against helminths may have been discovered. Normal human monocytes (Ellner and Mahmoud 1979) and mouse macrophages activated by lymphokine from concanavalin A-stimulated spleen cells (Bout, Joseph, David and Capron 1981) can kill schistosomula independently of antibody and complement.

Investigations of cytotoxicity against flukes have been hampered by the worm's continuous shedding of tegumental antigens (see Section 1.2.3). Bennett, Hughes and Harness (1980), Duffus and Franks (1980, 1981) and Sandeman (1980) have found that this causes cells to quickly detach from worms. Despite this, adherence to excysted metacercariae, juveniles and adults by peritoneal cells has been observed (Koie *et al.* 1977; Doy, Hughes and Harness 1980; Bennett *et al.* 1980; Eriksen 1980b; Davies and Goose 1981) and macrophages have been seen to attack damaged flukes (Davies and Goose 1981; Bennett *et al.* 1980). Adherence is a prerequisite for ADCC against schistosomula (Perez and Smithers 1977; Capron *et al.* 1975b; Kassis *et al.*, 1979a).

Duffus, Thorne and Oliver (1980) sidestepped the cytoadherence problem by purifying various effector proteins from eosinophils. One of these, lysozyme, which is possessed by macrophages, failed to damage excysted metacercariae. Another way of avoiding

this problem would be *in vivo* studies. A role for macrophages could be assessed using anti-macrophage serum or intraperitoneal lysosyme excretion and phagocytosis (Tanaka, Takaya, Kunimoto and Baba 1979).

Currently, evidence is sparse but it favours the suggestion that fluke survival is not greatly affected by macrophages whose roles appear to be confined to disposing of damaged worms and, perhaps, of stimulating fibrosis.

1.2.1.3 *Eosinophils*

Eosinophils, basophils/mast cells and neutrophils are the polymorphonuclear phagocytes. Eosinophilia has long been recognised as a symptom of helminth infection but only recently has it been proposed that eosinophils are the major effector cells in killing helminths (Kay 1978; David and Butterworth 1979; Soulsby 1979). The maturation of eosinophils from bone marrow precursors is T cell dependent. The properties of eosinophils (Kay 1978; Weller and Goetzl 1979) are very similar to those of neutrophils in that they release a similar battery of lysosomal enzymes, possess receptors for IgG and complement, are motile, attracted by histamine and kill the same range of microorganisms. However, eosinophils, unlike neutrophils, are attracted by the eosinophil chemotactic factor of anaphylaxis (ECF-A) tetrapeptides, Val/Ala-Gly-Ser-Glu (Goetzl and Austen 1975), release major basic protein (MBP) and an eosinophil-specific peroxidase (EPO). Also, eosinophil secretions antagonise some mast cell products (Goetzl 1976) which suggests that eosinophils have a homeostatic as well as killer role.

Eosinophils can phagocytose opsonised protozoans

(Mauel 1978) even though some shed antibody (Mitchell 1979a). In addition, they have been shown to kill by ADCC such parasites as trypanosomes (Sanderson, Lopez and Bunn Moreno 1977), *T. spiralis* (Grove, Mahmoud and Warren 1977; Kazura and Grove 1978; Taylor *et al.* 1979), *Trichostrongylus colubriiformis* (Gleich, Olson and Herlich 1979), and *S. mansoni* (Butterworth, Sturrock, Houba, Mahmoud, Sher and Rees 1975; Butterworth, David, Franks, Mahmoud, David, Sturrock and Houba 1977; Glauert and Butterworth 1977; Glauert, Butterworth, Sturrock and Houba 1978; M. Capron, Capron, Torpier, Bazin, Bout and Joseph 1978a; M. Capron, Rousseaux, Mazinque, Bazin and Capron 1978b; Kassis *et al.* 1979a).

Eosinophil mediated ADCC against schistosomula is

enhanced by the presence of complement (Ramalho-Pinto, McLaren and Smithers 1978; McLaren and Ramalho-Pinto 1979; James and Sher 1980) or mast cells (M. Capron *et al.* 1978a,b). Although antibody and complement alone can kill schistosomula (Clegg and Smithers 1972; Murrell and Clay 1972; Capron, Capron, Dupas, Bout and Petiprez 1974; Smith and Webbe 1974; Kassis, Warren and Mahmoud 1979b) the enhancement of killing by complement is thought to be due either to stimulation of eosinophils by the C5a component of complement (Kay, Shin and Austen 1973) or antibody-independent complement-mediated cytotoxicity (Tavares, Gazinelli, Mota-Santos and Dias da Silva 1978; Anwar, Smithers and Kay 1979; Ouaissi, Santoro and Capron 1980). M. Capron *et al.* (1978a,b) found that the effect of mast cell adherence to schistosomula via rat IgG_{2a} could be replaced by the mast cell protein ECF-A; showing that this mechanism

of enhancing eosinophil-mediated killing is also indirect.

Eosinophil adherence to schistosomula is via opsonins; IgG_{2a} in the rat (Capron, Dessaint and Capron 1978; M. Capron, Torpier and Capron 1979) and IgG₁ in the mouse (Ramalho-Pinto, de Rossi and Smithers 1979; James and Sher 1980). The cell then degranulates, releasing MBP, EPO and lysosomal enzymes. The parasite components exposed by the ensuing damage are phagocytosed (Glauert and Butterworth 1977; Glauert *et al.* 1978; McLaren, Ramalho-Pinto and Smithers 1978). However, MBP (Butterworth, Wassom, Gleich, Leogring and David 1979b) or EPO (Jong, Mahmoud and Klebanoff 1981) alone can kill schistosomula.

Doy *et al.* (1978) found marked eosinophil invasion of rat lamina propria within 8 h of a challenge with *F. hepatica* metacercariae, which is analogous to the response to schistosomula in mouse skin (Von Lichtenberg, Sher, Gibbons and Doughty 1976). The problem of creating conditions suitable for cytoadhesion and cytotoxicity (see Section 1.2.1.2) has been most clearly demonstrated by Duffus and Franks (1980) who cultured juvenile flukes with bovine granulocytes for up to one week. They found that adhesion by cells preceded their dislodgement rather than ADCC. Unfortunately, *in vitro* experiments that demonstrated antibody dependent cytoadhesion to flukes by eosinophils were of short duration (Goose 1978; Doy *et al.* 1980). However, electron microscopy of excysted metacercariae or adults after their temporary placement in the peritoneal cavity of immune rats has shown that the tegument is damaged after eosinophils flatten out and degranulate on it (Davies and Goose 1981; Bennett *et al.* 1980).

Neutrophils, macrophages and eosinophils were seen to attack the damaged flukes. Further evidence that eosinophils may spearhead the immune response is that bovine MBP alone can kill juvenile flukes *in vitro* (Duffus *et al.* 1980).

1.2.1.4 Other Cells

Considering the similarities between eosinophils and neutrophils it would be expected that if the former kill helminths then the latter do also. ADCC by neutrophils against schistosomula has been demonstrated by Dean, Wistar and Murrell (1974), Hsu, Hsu, Isacson and Cheng (1977) and Vadas, David, Butterworth, Pisani and Siongok (1979). However, neutrophils have not been implicated in resistance to challenge by liver flukes. Duffus and Franks (1980) found that this cell type can bind to flukes *in vitro*. However, a contrary result was obtained by Doy *et al.* (1980) and neutrophils, unlike eosinophils, are not prevalent in the immune rat gut wall (Doy *et al.* 1978). Thus, the role of neutrophils in fascioliasis is unclear.

Mast cells appear to be involved in immune responses to *N. brasiliensis* and coccidians (Ogilvie and Rose 1978; Wakelin 1978b; Soulsby 1979). Also, this granulocyte has been found to be capable of complement dependent (Sher 1976; Sher and McIntyre 1977) or IgG_{2a} dependent (Capron *et al.* 1978; M. Capron *et al.* 1978a, b) binding to schistosomula. The latter type of binding increases killing by eosinophils but neither mast cells nor their precursors, basophils, have been found to directly attack targets. Basophils and mast cells have been attributed with stimulatory roles early in delayed type hypersensitivity (DTH; Askenase 1977) and immediate type hypersensitivity

(Kay 1978). Fascioliasis involves DTH (Aalund and Nansen 1972; Aalund, Nielsen and Eriksen 1972; Vernes *et al.* 1972; Sinclair 1973; Flagstad and Eriksen 1974; Anderson, Hughes and Harness 1975). Moreover, large numbers of mast cells and globule leukocytes, which are derived from epithelial mast cells (Gregory 1979), have been found respectively in the lamina propria of immune rats (Doy *et al.* 1978) and the bile duct and intestinal epithelia of infected sheep (Campbell *et al.* 1978a; Knight 1980b). The possible involvement of these cells in resistance is worthy of study.

Two other cell types, K cells and NK cells, have not received attention with respect to their possible roles in fascioliasis. These lymphocytes are subpopulations of the null cells (Gupta and Good 1979b). Killer cells mediate ADCC against such targets as bacteria (Lowell, Smith, Artenstein, Nash and MacDermott 1979). The cytotoxicity of NK cells against tumours requires neither complement nor antibody (Lotzova and McCredie 1978; Kiessling and Wigzell 1979) but can be augmented by another tumour antagonist, interferon (Djeu, Huang and Herberman 1980). Eugui and Allison (1980) have argued that NK cells may be relevant to the survival of haemoprotozoans.

The widespread belief that antibody has a dominant role in resistance to schistosomes and liver flukes led to a lack of interest in leukocytes. This situation is being overturned now that individual cell types can be more readily identified and separated.

1.2.2 Antibody Responses

1.2.2.1 Detection

There are many methods by which antibodies against *F. hepatica* can be detected but most lack sensitivity or require high reagent concentrations and none are specific for flukes (see Sections 1.1.2 and 1.2.5).

Precipitation in tubes (Urquhart, Mulligan and Jennings 1954; Sewell 1964; Gundlach 1971a) and immunodiffusion (ID; Hughes 1962a; Sewell 1964; Geyer 1967; Cuperlovic, Sokolic and Movsesijan 1968; Gajos 1969; Sinclair and Kendall 1969; Doyle 1971; Gundlach 1971a; Flagstad *et al.* 1972; Deelder 1973; van Tiggele and Over 1976; Rajasekariah and Howell 1978b; Hillyer and Allain 1979; Haroun *et al.* 1980a) are simple, but insensitive, tests. The main advantages of ID are that it permits enumeration of precipitates and comparison of precipitation patterns.

Immunoelectrophoresis (IEP; Biguet, Rose and Havez 1960; Capron, Biguet, Tran van Ky and Rose 1964; Geyer 1967; Gajos 1969; Locatelli and Marzo 1969; Gundlach 1971a; Cuperlovic 1972a; Doyle 1973c; Choi and Lee 1979a,b) has the advantages of ID but is more sensitive (Biguet, Rose, Capron and Tran van Ky 1965c; Choi and Lee 1979a). The price for this added sensitivity is the need for highly concentrated antigens, of about 140 mg/ml (Biguet, Capron, Tran van Ky and D'Haussy 1962b; Deelder 1973). Compared with IEP, counterimmuno-electrophoresis (CEP; Hillyer 1975; van Tiggele and Over 1976; Hillyer and Santiago de Weil 1981) is rapid, uses less antigen to

give the same sensitivity but cannot detect cathodic antigens or properly enumerate antigens. The sensitivity of CEP is close to that of IEP (Hillyer 1975; van Tiggele and Over 1976) making it a valuable tool for immunodiagnosis (Hillyer and Allain 1979; Levine, Hillyer and Flores 1980). A different kind of immunoprecipitation method involves incubating freshly excysted metacercariae in sera (Wickerhauser 1961; Howell, Sandeman and Rajasekariah 1977) which is both a sensitive method of detecting antibody against antigens secreted by worms early in infection (Sandeman and Howell 1980, 1981b) and an effective way of collecting this antibody (Howell *et al.* 1977; Howell and Sandeman 1979; Sandeman and Howell 1981a). An antigen-antibody precipitate also appears around juvenile and adult worms cultured in the same way (Sandeman and Howell 1981a).

Flocculation (Benex 1964), miracidial immobilisation (Soulsby 1957; Movsesijan 1968; Rajasekariah and Howell 1978b), complement fixation (Minning and Vogel 1950; Benex, Lamy and Gledel 1959; Capron, Rose, Luffau, Biguet and Rose 1965; Cuperlovic *et al.* 1968; Gundlach 1971a), fluorescence on frozen sections of fluke (Thorpe 1965b; Coudert, Garin, Ambroise-Thomas, Kien Truong, Despeignes and Poithier 1967; Deelder 1973; Borojevic, Jovanovic and Movsesijan 1973; Movsesijan, Jovanovic, Aalund and Nansen 1975; Rajasekariah and Howell 1978b) or whole worms (Borojevic *et al.* 1973; Hanna and Jura 1977; Hanna 1980b), horseradish peroxidase labelling (Capron, Dugimont, Fruit and Bout 1975a; Oguz, Grelck, Tinar, Burgu and Alabay 1978) and the defined antigen substrate spheres (DASS) system (Deelder, Snoijnk and Ploem 1975; Petrovic and Deelder 1979) have also been employed in the study of fascioliasis.

Indirect haemagglutination (IHA) tests have been found to be as sensitive as IEP (Babenskas, Pagirays and Allisauskaite 1958; Biguet, Rose and Capron 1965b; Lang 1967; Cuperlovic *et al.* 1968; Gundlach 1971a; Movsesijan and Cuperlovic 1973; Deelder 1973; Hillyer and Capron 1976; Van Tiggele and Over 1976).

The enzyme linked immunoabsorbent assay (ELISA; Engvall and Perlmann 1972) has also been used in studies of fascioliasis (Burden and Hammet 1978; Hillyer and Santiago de Weil 1979; Ambroise-Thomas *et al.* 1980; Haroun *et al.* 1980b; Lehner and Sewell 1980). It (Levine *et al.* 1980) and, perhaps, radioimmunoassay (Gorrell and Howell 1981) are the most sensitive methods of antibody detection currently available.

1.2.2.2 Responses to Infection

The literature contains many examples of the rise and fall in antibody titres during a primary infection with *F. hepatica* (Capron *et al.* 1965; Sinclair and Kendall 1969; Movsesijan and Cuperlovic 1970, 1973; Movsesijan *et al.* 1975; Doyle 1971, 1973b, 1973c; Gundlach 1971b; Kendall and Sinclair 1971; Kendall *et al.* 1978; Borojevic *et al.* 1973; Deelder 1973; Van Tiggele and Over 1976; Hillyer and Del Llano de Diaz 1976; Hillyer and Allain 1979; Hillyer and Santiago de Weil 1979; Hanna 1980b; Haroun *et al.* 1980b; Levine *et al.* 1980; Lehner and Sewell 1980; Sandeman and Howell 1980, 1981a, 1981c; Duffus and Franks 1981). Titres peak about the time that flukes begin entering the bile ducts. After the worms are in the bile ducts titres gradually fall to low levels. This suggests that

antibody production is maximally stimulated when the greatest mass of worms are migrating in the liver and minimally stimulated when all worms are in the bile ducts. The suggestion that antibody levels bear a relationship to the size of the infection is supported by observations that antibody is detectable earlier in animals having heavier infections (Sinclair and Kendall 1969; Gundlach 1971b; Kendall and Sinclair 1971; Capron *et al.* 1965). However, contrary results have often been obtained and there does not appear to be a correlation between peak antibody levels and parasite numbers. The latter phenomenon may be the result of immunosuppression. Peak antibody titres during challenge infections rarely exceed those of primary infections and are often lower (Kendall and Sinclair 1971; Kendall *et al.* 1978; Gundlach 1971b; Rajasekariah and Howell 1978b; Haroun *et al.* 1980b; Duffus and Franks 1981; Sandeman and Howell 1981b) which further supports the contention that hosts are immunosuppressed (Sandeman and Howell 1981b).

The class of antibody most readily identifiable in these responses is immunoglobulin G (IgG). This class predominates in infected rats (Dargie *et al.* 1973; Howell and Sandeman 1979) and rabbits (Cuperlovic and Lalic 1970; Poluektova 1978; Ershov and Poluektova 1978). In infected sheep and cattle the bulk of antibody is of the IgG₁ class (Duffus and Franks 1981; Flagstad and Eriksen 1974; Movsesijan *et al.* 1975; Sandeman and Howell 1981c). The IgM response is up to 2 weeks ahead of the IgG response (Poluektova 1978; Cuperlovic and Lalic 1970; Duffus and Franks 1981; Sandeman and Howell 1981c). Even though the number of IgA secreting cells in bovine liver increases during infection (Flagstad and Eriksen 1974),

this isotype has been neglected. An increase in IgE levels is a feature of all helminth diseases (Jarrett and Urquhart 1971; Jarrett and Bazin 1974; Gupta and Good 1979a) but since it is difficult to detect it has not been closely studied with respect to fascioliasis (Jarrett 1972; Doyle 1973b; Bout, Dessaint, Dupas, Yarzbal and Capron 1977b; Ershov and Poluektova 1978). Perhaps interest will increase now that Capron *et al.* (1977) have shown that this isotype can act in concert with macrophages to kill schistosomes.

1.2.2.3 *Function*

The transfer of sheep, cattle or rat serum passively protects rats against infection (Armour and Dargie 1974; Dargie *et al.* 1973; Hayes *et al.* 1974b,c; Howell *et al.* 1977; Rajasekariah and Howell 1979). Experiments which failed to demonstrate passive transfer of resistance (Corba *et al.* 1971; Wickerhauser 1961; Doy *et al.* 1981) probably used serum containing insufficient amounts of antibody. Protective rat serum can be collected 7 weeks after infection (Hayes *et al.* 1974c). Earlier transfer has not been attempted.

The components of serum responsible for passive transfer of resistance appear to be IgG (Dargie *et al.* 1973; Howell and Sandeman 1979) and heat labile material (Doyle 1973b; Hayes *et al.* 1974c; Lang 1974) which could be IgE and/or complement. Doyle (1973b) gained evidence that reaginic antibody, possibly IgE, may have a role in expelling flukes from cattle.

In vitro killing of excysted metacercariae by immune serum such as occurs with schistosomula (see Section 1.2.1.3), has not been observed (Wickerhauser 1961; Howell *et al.* 1977; Hanna 1980a; Sandeman and Howell 1980; Duffus and Franks 1980, 1981; Chapman and Mitchell 1981b). However, excysted metacercariae incubated in immune serum from cattle, rats, sheep or mice, then placed in the peritoneum of a naive rat (Howell *et al.* 1977; Sandeman 1980), mouse (Lang 1974, 1976) or guinea pig (Wickerhauser 1961) are killed. Also, the precipitate formed on excysted metacercariae upon incubation in immune rat serum can be used to vaccinate rats against oral challenge (Howell 1979; Howell and Sandeman 1979). These findings point to a mechanism of *in vivo* killing that includes both antibody and cells and is perhaps similar to schistososome killing (reviewed by Phillips and Colley 1978; Cox 1979; David and Butterworth 1979; Capron, Dessaint and Capron 1980; Houba 1980).

1.2.3 *Evasion of Immune Responses*

The trematode tegument appears to be the most obvious site for immune attack and repulsion of that attack by the parasite. It would be expected that digestive enzymes would destroy antibodies and leukocytes in the caeca. Indeed, Dawes (1963) observed that flukes ingest leukocytes.

The tegument

A turnover rate of tegumental antigens of about 2 to 4 h appears to be a means whereby schistosomes keep the immune response at bay (Kusel and Mackenzie 1975; Kusel, Mackenzie and McLaren 1975;

Kusel, Sher and Perez 1975; Wilson and Barnes 1977, 1979; Kemp, Brown, Merritt and Miller 1980; Samuelson, Sher and Caulfield 1980). A similar kind of mechanism may also be used by liver fluke (Howell and Sandeman 1979; Hanna 1980a, b; Duffus and Franks 1980, 1981).

The tegument, covered by the carbohydrate-rich glycocalyx, consists of a layer of cytoplasm with cytoplasmic bridges that extend down through the 2 muscle layers to bulbs of nucleated cytoplasm (termed tegumental cells). The tegument has many roles (Lumsden 1975; Erasmus 1977) including uptake of nutrients such as glucose and amino acids (Pappas and Reed 1975; Chappell 1976), excretion (Pantelouris 1965), ion regulation (Mettrick and Podesta 1974) and secretion of glycoproteins (Hanna 1980c). Its structure has been examined in detail (Bennett and Threadgold 1973, 1975; Hanna and Threadgold 1976; Threadgold 1976; Davies 1978; Threadgold and Brennan 1978). There are **2** types of tegumental cells which synthesize the glycoprotein secretory bodies T_0 , T_1 and T_2 . The predominant cell type changes during fluke development, as does the type of secretory body on the apical membrane, but these 2 events are asynchronous (Bennett and Threadgold 1975; Bennett 1978; Hanna 1980c).

Immunodepression

Immunodepression is the phenomenon of an immune system responding weakly to an antigenic stimulus. This should be distinguished from immunosuppression, which is the active process of lowering an immune response (Terry 1978).

Once flukes enter the bile ducts titres of tegument-binding antibodies begin to fall (Thorpe 1965b; Hanna and Jura 1977; Hanna 1980b; Sandeman and Howell 1980; Duffus and Franks 1981). The change to expressing T₂ bodies on the tegument at this time (see Section 1.2.5) may be a cause of this immunodepressed state. Alternatively, or perhaps coincidentally, the immunogenicity of flukes may decrease as occurs in schistosomes by a mechanism that is independent of immunosuppression and the host antigen phenomenon (Dean 1977; Moser, Wassom and Sher 1980; Snary, Smith and Clegg 1980; Abbas, James and Sher 1981). Diminished contact between flukes and the immune system may also be involved. The rapid cell mediated death of adults upon transfer to the peritoneum 6 weeks but not 12 months after infection (Hughes *et al.* 1977) supports all 3 views.

This question could be further investigated by looking at the amounts and types of circulating antigens during infection. The results of these experiments might not have a simple explanation. Adult antigens meet the host via mucous membranes of the bile ducts and intestine. The gut is known to selectively absorb antigens (Abrahamson, Powers and Rodewald 1979; Williams 1979). If fluke antigens are selectively absorbed, this could influence the nature of the immune response against adults. Antigens the gut is exposed to in small amounts over long periods can induce systemic tolerance (Tomasi 1980), while in the gut antibodies are produced which sometimes exclude most of the antigen from absorption (immune exclusion; Walker and Isselbacher 1977). These 2 phenomena - tolerance and selective immune exclusion - may account for the state of concomitant immunity (Gerhson, Carter and Kondo 1967; Smithers, Terry and Hockley 1969)

in rats in which adult flukes survive despite the destruction of juveniles as they penetrate the gut wall and invade the liver (Hayes *et al.* 1972, 1973, 1974a; Hayes and Mitrovic 1977; Hayes 1978; Rajasekariah and Howell 1977a, 1977b, 1978b, 1979, 1980, 1981; Campbell *et al.* 1978b; Doy *et al.* 1978; Kelly, Campbell and Dineen 1980).

The fall in antibody levels that approximately coincides with patency could have other causes such as inadequate nutrition (Dimitrov, K'ncheva and Georgiev 1978; Gross and Newberne 1980; Sykes, Coop and Rushton 1980a) and immunosuppression. Immunosuppression occurs in a wide range of parasitic diseases (Terry 1978; Warren 1978b; Bloom 1979; Mitchell 1977, 1979a, b) including schistosomiasis (Pelly, Ruffier and Warren 1976; Dessaint, Camus, Fischer and Capron 1977; Mota-Santos, Tavares, Gazzinelli and Pellegrino 1977; Cottrell, Humber and Sturrock 1980a; Cottrell, Sturrock and Vanhoegaerden 1980b; Ottesen and Poindexter 1980; Trizio, Della Bruna and Isetta 1980). Parasite products (Mitchell 1979a; Albright and Albright 1981) and circulating immune complexes (Kano and Milgrom 1980; Theofilopolous and Dixon 1979; Cottrell *et al.* 1980b; Ottesen *et al.* 1980) have been implicated as causes. Circulating antigens have been found in fluke infected cattle and humans (Ambroise-Thomas *et al.* 1980; Robert *et al.* 1980). However, the presence of immune complexes in fascioliasis has not been demonstrated but can be deduced from findings (Locatelli and Simonic 1974; Sandeman and Howell 1981b) that suggest the manifestation of immune complex disease (WHO 1977; Andres 1979; Houba 1979; Hutt 1980).

Flukes may interfere with immune responses in other ways. High concentrations of soluble protein have been found to inhibit eosinophil function (Jong, Henderson and Klebanoff 1980). Such a situation could be expected to occur on the fluke tegument where glycoproteins are continually secreted.

Proteases

Many leukocytes near flukes *in vivo* are dead which may be the result of physical disruption by the oral and ventral suckers (Dawes 1963) or due to secreted toxins such as proteolytic and other digestive enzymes (Thorsell and Björkman 1965; R. Howell 1966; Locatelli and Beretta 1969; Locatelli and Paoletti 1969; Howell 1973; Goose 1978; Hajdu, Matskasi and Juhasz 1979; Simpkin, Chapman and Coles 1980). One fluke protease has been shown to cleave Ig (Chapman and Mitchell 1980; Chapman and Mitchell 1981b). A similar protease possessed by schistosomes inhibits ADCC by macrophages because it splits Ig molecules into their Fab and Fc components (Auriault, Joseph, Dessaint and Capron 1980; Auriault, Ouaisi, Torpier, Eisen and Capron 1981).

Host antigens

Ig can be found on the surface of juvenile *F. hepatica* taken from mice (Howard *et al.* 1980). It is possible that some of this antibody is attached via the Fc region to Fc receptors on the tegument as occurs on the schistosome tegument (Torpier, Capron and Ouaisi 1979; Santoro, Ouaisi, Pestel and Capron 1980a; Tarleton and Kemp 1981). Immune complexes have been shown to block cytotoxicity against schistosomes probably by binding to these Fc receptors (M. Capron *et al.* 1980).

However, Hanna (1980b) showed that Fab fragments and intact Ig molecules bind equally well to whole flukes and Howard *et al.* (1980) could not detect Ig on flukes removed from athymic mice. Therefore, the tegument of *F. hepatica* is unlikely to possess Fc receptors.

Schistosomes also possess receptors for the complement components Cl_q (Santoro *et al.* 1980a) and C3 (Tarleton and Kemp 1981) which, like Fc receptors, are thought to bind these host antigens to the worm's surface to block or sterically hinder antibody binding. There are as yet no similar studies on *F. hepatica*.

Many other host antigens, such as transplantation antigens (Sher, Hall and Vadas 1978), α -2 macroglobulin (Damian, Greene and Hubbard 1973; Kemp, Damian, Greene and Lushbaugh 1976), Forssmann antigen (Dean and Sell 1972) and blood group antigens (Dean 1974; Goldring, Clegg, Smithers and Terry 1976; Goldring, Kusel and Smithers 1977) have been found on the schistosome tegument. It has been postulated that these host antigens, like immune complexes, shield schistosomes from immune attack (Smithers *et al.* 1969; Smithers 1976). However, the only evidence to support this postulate is indirect and attempts to repeat it have failed. In support Tavares, Cordeiro, Mota Santos and Gazinelli (1980) found that the antigenicity of schistosomula maintained *in vitro* decreased only if serum (as a source of host antigens) was included in the medium. The converse, showing altered antigenicity independent of host molecules, was obtained by Dean (1977), [Samuelson *et al.* (1980) and Dessein, Samuelson, Butterworth, Hogan, Sherry, Vadas and David (1981). Like the schistosomes, the liver fluke shares some antigens with its hosts (see Section 1.2.5) but these antigens do not appear to be presented to the host by living worms (Hughes and Harness

1973a, b) and they are therefore unlikely to be involved in evading host immune responses.

Complement depletion

Some cestodes (Kassis and Tanner 1976, 1977; Hammerberg, Musoke and Williams 1977; Hammerberg and Williams 1978; Hammerberg, Dangler and Williams 1980; Perricone, Fontana, De Carolis and Ottaviani 1980) and schistosomes (Santoro, Bernal and Capron 1979; Santoro, Pestel, Le Presle, Liebart and Capron 1980b; Van Egmond, Deelder and Daha 1981) contain molecules that deplete complement by nonimmunological activation. Hydatid fluid contains a polyhexosamine ceramide complex that is anticomplementary. A similar molecule in flukes does not have this activity (Hrzenjak, Muic and Ehrlich 1979). There appear to have been no other attempts to demonstrate anti-complementary activity in *F. hepatica*.

Thus, it seems that the major defences against immune responses used by *F. hepatica* are to shed antigens continuously from its surface and enzymatically digest immune effectors such as antibodies.

1.2.4 *Vaccination*

The eventual objective of research on vaccination against fascioliasis is to discover a protocol which will confer on sheep, particularly, the persistent ability to resist infection. This goal is clearly long-term.

Vaccination studies have mainly used rats but some experiments have used cattle or rabbits. These experiments will be discussed together but those that involved mice or sheep will be treated separately.

Adult fluke antigens

With few exceptions (Kerr and Petrovich 1935; Sinclair and Joyner 1974), no animals have been protected against homologous challenge using dead vaccines (homogenate or incubation products) derived from adult flukes (Urquhart *et al.* 1954; Healy 1955; Ershov 1959; Hughes 1962a; Lalic, Cuperlovic and Movsesijan 1976; Lehner and Sewell 1979; Rajasekariah *et al.* 1979b; Burden and Hammet 1980; Hughes, Harness and Doy 1981). Not surprisingly, dead adult flukes transplanted into the peritoneal cavity of naive rats do not elicit a protective immune response (Haroun *et al.* 1980a). However, implanted live adults protect rats against oral challenge if present for as little as 2 weeks (Eriksen and Flagstad 1974; Anderson *et al.* 1975; Haroun *et al.* 1980a). Paradoxically, adults inside diffusion chambers appear able to stimulate immunity (Haroun *et al.* 1980a) while incubation products of the same life stage, which would include soluble egg antigens, do not (Lehner and Sewell 1979; Rajasekariah *et al.* 1979b; Burden and Hammet 1980). Perhaps the parasite molecules the host is exposed to *in vivo* are dissimilar to those released *in vitro*. Experiments by Movsesijan and Borojevic (1973) and Lehner and Sewell (1980) have shown that fluke antigens in the bile are indistinguishable from those collected *in vitro* but this may be because they are equally degraded.

The contribution of eggs to the immune state induced by implanted adults has been debated. The experiment by Haroun *et al.* (1980a)

may indicate that soluble egg antigens elicit immunity. There is no evidence that fluke eggs release antigens except by inference from schistosome research (Houba 1980). Rajasekariah and Howell (1978a) found fluke eggs to be an effective vaccine but Burden and Hammet (1980) did not. Christensen, Monrad, Nansen and Frandsen (1980) found that mixed sex, but not single sex, mature infections of schistosomes protect mice against flukes which supports the contention that eggs are involved in immunity.

Antigens of invasive stages

Like implanted adults, implanted encysted metacercariae (Kelly and Campbell 1979; Rajasekariah and Howell 1978a), excysted metacercariae (Hughes *et al.* 1981) and juveniles (Rajasekariah and Howell 1978a; Hughes *et al.* 1981) each induce protection against challenge within 14 d. Interestingly, vaccination using metacercariae attenuated by γ or x-irradiation and administered *per os* requires the survival of migrating juveniles for greater than 10 d (Dawes and Hughes 1964; Boray 1967b; Thorpe and Broome 1972; Armour and Dargie 1974; Dargie *et al.* 1974; Nansen *et al.* 1975; Hughes *et al.* 1981). It appears that the host requires 11 to 14 d exposure to some component(s) of living worms in order to become immune.

Excretory/secretory antigens of the early life stages can be used in vaccines. Cattle have been protected against oral challenge by injections of 16 d-old worm incubation products (Hall and Lang 1978). Davies, Rickard [Smyth and Hughes (1979)] were unable to protect rats against challenge given 5 weeks after a single subcutaneous injection of small amounts of

metacercarial incubation products. However, if what is presumed to be the same antigen is immunoprecipitated by incubating excysted metacercariae in immune rat serum, it confers protection on naive recipients (Howell 1979; Howell and Sandeman 1979). The immunoprecipitate probably contains a few major antigens (Howell and Sandeman 1979; Sandeman and Howell 1980, 1981a). Immunoprecipitation of this antigen may have been important for stimulating immunity for two reasons. Firstly, antigens presented as immune complexes are more immunogenic than antigen alone (Dennert 1971; Laissue, Cottier, Hess and Stoner 1971; Diener and Feldmann 1972; Houston, Pedersen, Cole and Spertzel 1974). Secondly, the antibody molecules may protect their corresponding antigenic determinants from denaturation.

The observations that implantation of any life stage from metacercariae to adult protects rats against fluke infection suggest a high degree of antigenic conservation. Indeed, direct demonstrations of this conservatism have been made (Dodd 1969; Gorrell 1977; Irving and Howell 1982a; Sandeman and Howell 1981a). Antigenic changes during fluke development appear to be restricted to the tegument (Thorpe 1965a; Bennett 1978; Davies 1978; Hanna 1980b; Duffus and Franks 1981). Classified on the basis of tegumental cell types (see Section 1.2.3), there are three fluke life stages: excysted metacercariae, juveniles and adults. The apical membrane of excysted metacercariae presents T_0 granules to its environment. T_1 bodies start to appear 2 d after excystment but the change is not complete until day 5. T_2 bodies are expressed on the surface after flukes enter the bile duct. The relationship of these granules to the various antigens that have been detected

in *F. hepatica* has not yet been established. However, the granules are thought to be antigenic and directly involved in diverting immune attack (Bennett *et al.* 1980; Hanna 1980b; see Section 1.2.3).

Heterologous antigens

Attempts to induce immunity against fascioliasis have included the use of some heterologous antigens. An approach similar to that of Alger and Harant (1976) in using mosquito antigens to vaccinate mice against *Plasmodium berghei* has been applied by Kozar (1974). She injected rats with intermediate host antigens (*L. tomentosa* or *Galba truncatula*); these induced protection against challenge.

Vaccination against flukes has been achieved by prior infection with *N. brasiliensis* (Doy *et al.* 1981), *S. mansoni* (Christensen, Nansen, Frandsen, Bjorneboe and Monrad 1978; Christensen *et al.* 1980), *Taenia hydatigena* (Campbell, Kelly, Townsend and Dineen 1977; Dineen, Kelly and Campbell 1978) and *S. bovis* (Sirag, Christensen, Nansen, Monrad and Frandsen 1981) but not by simultaneous infection with *Ostertagia ostertagia* (Burden, Hughes, Hammet and Collis 1978). Unfortunately, the experiments with *T. hydatigena* have not been supported by later work (Hughes, Harness and Doy 1978; Campbell, Dineen and Kelly 1979; Rajasekariah, Rickard, Montague and Mitchell 1979a).

Sheep and mice

Although sheep can be vaccinated against helminth species such as *T. hydatigena* (Onawunmi and Coles 1980; Gemmell and Johnstone 1981), *T. ovis* (Rickard and Bell 1971; Rickard and Adolf 1977), *Trichostrongylus colubriiformis* (Dineen, Gregg and Lascelles 1978), *S. mattheei* (Taylor, James, Nelson, Bickle, Dunne and Webbe 1976; Dargie, Berry, Holmes, Reid, Breeze, Taylor, James and Nelson 1977) and *S. bovis* (Taylor, James, Bickle, Doenhoff and Nelson 1978) various attempts to do the same with fascioliasis have failed (Boray 1967b; Campbell *et al.* 1978a; Kelly and Campbell 1979; Sandeman, Howell and Campbell 1980). However, there are some exceptions. Ross (1967c) and Dargie *et al.* (1974) have observed resistance to challenge following intramuscular implantation of adult flukes or infection with γ -irradiated metacercariae. Other evidence that at least some flocks may be able to damage flukes comes from reports that challenge infections are retarded (Sinclair 1971a, 1973; Rushton 1977; Campbell *et al.* 1978a). Furthermore, serum from infected sheep is lethal to flukes if injected into rats at the time of infection (Armour and Dargie 1974; Rajasekariah 1977; G.B. Mitchell, Armour, Ross and Halliday 1981) or excysted metacercariae are incubated in it before their transfer to naive rats (Sandeman 1980). These observations suggest that sheep lack, or are defective in, some component of their immune system that is necessary for the elimination of flukes, or that the parasite blockades immune responses in this host (see Section 1.2.3). This defect may be overturned by some unusual circumstances such as the veterinary history of the flock, nutrition or genetics of host or parasite (see Section 1.1.1) or the type of immunogen so that the immune response becomes lethal to flukes.

Murine responses to infection parallel those of sheep (see Section 1.2). Mice can be vaccinated against helminths such as *Mesocostoides corti* (Kazacos 1976), *T. taeniaeformis* (Rajasekariah, Rickard and Mitchell 1980) and schistosomes (Taylor *et al.* 1978; Bickle, Taylor, Doenhoff and Nelson 1979; Hillyer 1980) but not *F. hepatica* (Hughes 1962b; Dawes and Hughes 1964; Gold and Lengy 1972; Harness *et al.* 1977; Lehner and Sewell 1979; Rajasekariah *et al.* 1979b). However, the mouse seems to affect flukes sub-lethally in that challenge flukes migrate to the bile duct faster than primary flukes (Harness *et al.* 1976, 1977). In addition, it may be that some mice can resist a challenge infection (Lang and Hall 1977).

Novel approaches to modifying the sheep immune response such as selective breeding, the use of new adjuvants and negation of parasite-induced blockade of the immune response might be found from further studies on mice.

Conclusion

All life stages from metacercariae to adult appear to be potential vaccine sources. The failure of most dead vaccines may simply be due to the degradation of antigens that stimulate protective immune responses (functional antigens) or antigenic competition (Jerne, Henry, Nordin, Fuji, Koros and Lefkovits 1974; Cocito, Michot, Radovich and Talmage 1979) in a mixture of antigens. A solution to the latter is to identify and make use of the functional antigens. Attention will then be focussed on methods of producing sufficient amounts of antigen for large scale vaccination using hybridoma technology (Pearson, Pinder, Reolants, Kar, Lundin, Mayor-

Withey and Hewett 1980), cell fusion (Howell 1981) or recombinant DNA technology.

1.2.5 *The Antigens of F. hepatica*

The study of small populations of the many antigens possessed by flukes (Hillyer and Cervoni 1978) remains embryonic and the list of purified antigens is short but some useful information has emerged, particularly with respect to the tegument.

Serology

The major use of fluke antigen preparations has been in serology. Delipidised supernatants of homogenised adult worms (Kent 1960; Movsesijan and Borojevic 1973) have been the most commonly employed because they are the most readily obtained in quantity. Removal of the lipid layer after centrifugation of the homogenate imparts greater sensitivity to immunodiffusion, but not indirect haemagglutination tests (Cuperlovic *et al.* 1968; Gundlach 1971a). Another characteristic of this antigen preparation is that most of the polysaccharide component is in high molecular weight molecules (Choi and Lee 1979b). The next most easily obtained and commonly used antigen is the incubation products of adults. This antigen preparation has been found to be more suitable than somatic antigens for examining the serology of prepatent infections (Minning, Newsome and Robinson 1958; Gundlach 1971a). However, this observation was not repeated in later work (Rajasekariah and Howell 1978b; Lehner and Sewell 1980). The antigens in incubation products almost

all contain polysaccharide and are glycoproteins (Gundlach 1971a); all are present in preparations of somatic antigen (Gundlach 1971a; Movsesijan and Borojevic 1973; Gorrell 1977).

Innumerable reports of crossreactivity between helminth antigens attest to the central problem of immunodiagnosis. The degree of antigen sharing appears to be correlated with phylogenetic relatedness (Biguet, Capron and Tran van Ky 1962a; Biguet *et al.* 1962b; Capron, Biguet, Vernes and Afchain 1968). For example, antigen sharing is greatest (5 antigens) with the schistosomes (Capron *et al.* 1968; Pelley and Hillyer 1978; Petrovic and Deelder 1979). This crossreactivity can be removed by selecting appropriate gel filtration fractions (Hillyer and Capron 1976; Hillyer and Santiago de Weil 1977, 1979, 1981; Choi and Lee 1979a,b). Other gel filtration experiments (Biguet *et al.* 1965c; Cuperlovic 1972a,b; Cuperlovic and Lalic 1972; Movsesijan and Borojevic 1973; Lehner and Sewell 1980; Choi and Lee 1979a) have attempted to enumerate the antigens in each peak by IEP. There are fewer antigens in high molecular weight fractions.

Another method of increasing the specificity of immuno-diagnostic reagents has been to remove crossreacting antigens or antibodies by absorption. For example, sera from patients with hydatidosis and fascioliasis crossreact because both parasites contain the human P1 blood group antigen (Damian 1964; Ben-Ismail, Carme and Gentilini 1979). The crossreactivity can be removed by incubating sera from patients who have the P2 blood group with P1 red cells

(Ben-Ismail, Carme, Niel and Gentilini 1980; Ben-Ismail, Rouger, Carme, Gentilini and Salmon 1980).

C substance in helminths is another source of serological false positives because it forms 2 precipitates with C reactive protein (CRP) that animals produce during any infection, including fascioliasis (Capron *et al.* 1964, 1965; Biguet, Capron, Tran van Ky and Rose 1965a; Geyer 1967; Doyle 1973c). These precipitates can be removed by washing IEP gels in trisodium citrate (Biguet *et al.* 1965a).

Fraction II

Fraction II (Tailliez and Korach 1970a) is an antigen preparation that has been used to overcome serological crossreactions.

Tailliez and Korach (1970a) removed the lipoprotein component of an adult fluke soluble somatic extract with dextran sulphate and CaCl_2 . They then used gel filtration and potassium phosphate precipitation to prepare fraction II and preparative electrophoresis to separate it into subfractions IIa, IIb, IIc₁ and IIc₂. These subfractions are glycoproteins of about 42 kilodaltons. They were divided into two groups, IIab and IIc, on the bases of electrophoretic mobility, amino acid composition and antigenic analysis. Even so, IIab and IIc partly crossreact (Tailliez and Korach 1970a).

When reacted in IEP with sera from infected or immunised animals fraction II forms a precipitate called arc 2 (Biguet *et al.* 1962a; Capron *et al.* 1964; Capron *et al.* 1965; Capron, Vernes and Fruit 1971) that is genus specific (Tailliez, Mangalo and Korach 1967; Tailliez and Korach 1970b). The genus *Fasciola* includes *F. gigantica*, which has not been distinguished from *F. hepatica* antigenically (Sewell 1964). Wattre *et al.* (1978) considered the presence of arc 2 to be an accurate diagnostic test for fascioliasis in humans.

Circulating antigens

Since fraction II resides in the tegument (Tailliez and Korach 1970b; Hillyer 1980) it is not surprising that it can be found in the blood, bile and faeces of some infected cattle (Robert *et al.* 1980). In Section 1.1.2 it was pointed out that circulating antigens may be useful diagnostic tools. Schistosomiasis results in the appearance of 2 antigens (anodic antigen and cathodic or 'M' antigen) in the serum, urine and milk of humans (Carlier, Bout, Bina, Camus, Figuerido and Capron 1975; Carlier, Bout and Capron 1978; Carlier *et al.* 1980; Hillyer, Almenas and Knight 1976; Madwar and Voller 1975; Santoro, Carlier, Borojevic, Bout, Tachon and Capron 1977; M. Capron *et al.* 1980; Deelder, Kornelis, van Marck, Eveleigh and van Egmond 1980) and animals (Berggren and Weller 1967; Gold, Rosen and Weller 1969; Bawden and Weller 1974; Nash, Prescott and Neva 1974; Deelder, Klappe, van den Aardweg and van Meerbeke 1976; Houba, Koech, Sturrock, Butterworth, Kusel and Mahmoud 1976; Carlier *et al.* 1978; Ferreira, Caldini, Hoshino-Shimizu and Camargo 1979; Santoro, Vandemeulebroucke and Capron 1979).

These antigens are produced by caecal cells, are mainly carbohydrate and are also present as immune complexes. The tegument and eggs also release antigens but these are not important for immunodiagnosis (Wilson 1978; Houba 1980). The poor correlation between faecal egg counts and circulating fraction II in cattle (Robert *et al.* 1980) suggests that this may also be true with respect to fascioliasis. If this is so, it might be that some of the poorly defined caecal antigens of *F. hepatica* (Simpkin *et al.* 1980) will prove to be diagnostic tools.

Purification of shared antigens

Interestingly, the most thorough purification of fluke antigens has been of those that crossreact with *S. mansoni* because it was found that these antigens confer protection against schistosomiasis on mice and hamsters (Hillyer, Del Llano de Diaz and Garcia-Blanco 1975; Hillyer 1976; Hillyer, Garcia-Blanco and Del Llano de Diaz 1976; Hillyer, Del Llano de Diaz and Reyes 1977). The *in vitro* antigenicity of these antigens can be augmented by 0.03% sodium dodecyl sulphate (SDS) (Hillyer, Pelley and Del Llano de Diaz 1979) but lessened by higher concentrations of SDS or pH 3 buffers (Hillyer and Ramos 1980). Purification was achieved with affinity chromatography followed by gel filtration with Sephadex G200. This yielded a preparation, Fh_{smIII}, which was shown by 2-dimensional immunoelectro-diffusion (2-D IED) using an homologous antiserum, to contain 6 antigens. The largest antigen-antibody precipitate (denoted Fh_{smIIIM}) in this gel was cut out and injected into rabbits. The resulting antiserum was used to purify Fh_{smIIIM} to homogeneity by affinity chromatography (Hillyer and Cervoni 1978). Fh_{smIIIM} confers upon mice immunity against schistosomiasis (Hillyer 1979). This antigen was found to

bind Concanavalin A sepharose and have an isoelectric point of about 4 (Hillyer and Sagramoso de Ateca 1979). It has been localised in the tegument (Hillyer 1980).

Host antigens

Crossreactions between animals as close phylogenetically as the various groups of helminths are to be expected. That flukes share antigens with various host and non-host mammals (Capron *et al.* 1968; Gajos 1969; Movsesijan and Borojevic 1973; Gorrell 1977; Bennett 1978; Ben-Ismaïl *et al.* 1979; Lehner and Sewell 1980) and molluscs (Converse 1968; Deelder 1973; Kozar 1974; Gorrell 1977) might also be expected when one considers that these animals have many basic physiological similarities. Moreover, the extent of this antigen sharing appears to reflect the phylogenetic relatedness of these animals rather than the evolution of fluke antigens which mimic those of its hosts (Gorrell 1977). Most of the mammalian antigens in flukes are partly digested meals because the flukes have not normally been permitted to regurgitate their gut contents before somatic or secreted antigens were prepared (Movsesijan and Borojevic 1973; Gorrell 1977). The significance of other shared antigens is discussed in Section 1.2.3.

Other purification studies

Simpkin *et al.* (1980) purified, by ethanol precipitation and gel filtration, a digestive protease from the regurgitated caecal contents of adult flukes. This protease, having an apparent molecular weight of 12,000, preferentially cleaves globin and haemoglobin. It is not immunogenic during infection. This is probably fortuitous because an antigen released in such large amounts as this protease

(10 mg/g per day) would be likely to induce immune complex disease (Theofilopolous and Dixon 1979; Hutt 1980; Simpkin *et al.* 1980).

Other purification work includes that of Behm and Bryant (1980) who purified the enzyme pyruvate kinase by physicochemical methods and of Yoshihara, Suzuki, Suto and Fujita (1979) who raised monospecific antisera to 2 fluke antigens by injecting rabbits with immunoprecipitates obtained by immunodiffusion. Yoshihara *et al.* (1979) found that one antiserum bound tegumental cells and the other bound excretory ducts.

Bout *et al.* (1977b) have shown that one of 2 allergens discovered to be in adults forms a precipitate with infected serum similar to arc 2. However, Bout *et al.* (1977b) determined that both allergens are lipoproteins and overlooked the finding by Tailliez and Korach (1970a) that fraction II contains no lipid. The other lipoprotein allergen appears to be the same antigen that Korach and Benex (1966a,b) isolated. This antigen is nearly 50% lipid but the lipid moiety was only antigenic while present as lipoprotein. However, some other non-protein molecules of *F. hepatica* are antigenic (Minning and Fuhrmann 1955; Sewell 1964; Hillyer and Sagramoso de Ateca 1979) which needs to be considered in antigenic analyses.

Conclusion

Few isolated fluke antigens have been studied and most are poorly characterized. Separation of antigens on the basis of molecular weight, charge and solubility in various chemicals has

limitations, the methods are tedious and readily applied to few antigens. Analysis of single antigens using monospecific antisera is limited to those antigens that are able to be purified by physicochemical means and/or are separable in immunodiffusion gels. These appear to be reasons for the neglect of caecal, circulating and egg antigens. There is a clear need for new approaches to antigenic analysis. One such approach exploits hybridoma technology.

1.3 HYBRIDOMA TECHNOLOGY

Antigenic complexity is a problem encountered not only by immunoparasitologists but in most fields of biology. For this reason numerous methods have been developed for narrowing the range of antigens being studied. "Specific" antisera having minimal crossreactivity with non-target antigens are frequently employed in these endeavours. The time and difficulties involved in attempting to produce monospecific antisera by immunizing animals are great. Usually, the antigen needs to be partially purified, a number of injections are required and crossreactivity needs to be removed by absorption. The result is an isotypic mixture of an often small, always finite quantity of monospecific antiserum. This leads to problems of standardisation that are particularly pronounced in tissue typing.

This situation has changed dramatically since Köhler and Milstein (1975) discovered a means of producing monoclonal antibodies (MCA) of predetermined specificity using what has since become known as hybridoma technology.

1.3.1 *Theory*

The clonal selection hypothesis (Burnet 1959) predicts that each antibody-forming cell (AFC) expresses a single, invariable antigen specificity (idiotype). This prediction has been shown to be true (Klinman 1971; Nossal 1978). A method exploiting this fact in order to obtain MCA involved culturing individual pieces of spleen so that each piece was likely to contain only one AFC clone, a technique

referred to as the spleen fragment method (Klinman 1969, 1971; Gerhard, Braciale and Klinman 1975; Levy and Dilley 1977; Sigal and Klinman 1978). Another approach is to transform B cells in order to produce cell lines that secrete MCA. Mouse B cells can be transformed by Abelson murine leukemia virus (A-MuLV; reviewed by Raschke 1980) but this technique has limited application and specific antibody is not often obtained. The similar use of Epstein-Barr virus (EBV) on human B cells has had much greater, but limited, success (see Section 1.3.5).. Unfortunately, using any of these methods, clones rarely survive longer than a month and therefore secrete relatively little antibody.

The approach used by Köhler and Milstein (1975, 1976) was to fuse AFCs from a sheep red blood cell (SRBC)-immunized mouse with myeloma cells. This yielded hybrid cells which could be cloned to become established cell lines (hybridomas) that secreted MCA specific for SRBC. Thus, it was shown that an AFC could be immortalised by fusing it with a malignant cell.

Although most cells are capable of spontaneous fusion with any other cell (Bernhard 1976; Ringertz and Savage 1976), various procedures can increase the fusion frequency. These procedures include the use of fusion agents such as Visna virus, Sendai virus or polyethylene glycol (PEG) and immunization of the AFC donor. Also, it was found that the yield of antibody-secreting hybrids is greatest if a malignant B-cell line (myeloma), rather than any other tumour cell, is used for fusion (Schwader and Cohen 1973; Köhler, Pearson and

Milstein 1977; Hämmerling, Reth, Lemke, Hewitt, Melchers and Rajewsky 1978). The myeloma needed to be selected for its ability to fuse with AFCs.

Originally, the P3/X63 Ag8-1 mouse myeloma, selected from the P3 (MOPC21) line (Horibata and Harris 1970; Potter 1972), was used but later a variant that does not secrete Ig (P3/NSI-1 Ag4-1) was isolated (Cowan, Secher and Milstein 1974).

The frequency of reversion to non-secretion of Ig by X63 cells, which secrete IgG₁ molecules and free κ light chains, is 10^{-3} to 10^{-4} per cell per generation (Cotton, Secher and Milstein 1973; Cowan *et al.* 1974). A hybrid cell parented by an X63 cell and an AFC secretes Ig molecules in which heavy and light chains of both the AFC and myeloma parents are randomly assorted. Therefore, only 1/16 of the secreted antibody binds antigen. This problem is lessened by fusing with NSI cells because their hybrid progeny secrete only myeloma derived κ light chains bound to AFC derived heavy chains and intact Ig molecules derived from the AFC (Köhler *et al.* 1976; Springer, Galfre, Secher and Milstein 1978). The X63 derived Sp2/0 Ag14 line is superior to the X63 and NSI lines for it synthesizes neither heavy nor light chains of its own (Schulman, Wilde and Köhler 1978). A variant of Sp2/0, FO (Fazekas de St Groth and Scheidegger 1980), has half the generation time and could replace its parent. Other X63 derived myelomas include P3/X63 Ag8 U1 which is similar to NSI (Yelton, Diamond, Kwan and Scharff 1978) and P3/X63 Ag8.653 which is similar to SP2/0 (Kearney, Radbruch, Liesegang and Rajewsky 1979).

Myelomas derived from cell lines other than MOPC21 are also available for fusion with AFCs. These include PuBul-Ou which secretes IgG_{2a} (Köhler *et al.* 1976), MPC11-45.6 TG 1.7 which secretes IgG_{2b} (Margulies, Kuehl and Scharff 1976).

1.3.2 Fusion

The various methodologies used for cell fusion are mostly based on those of Galfre, Howe, Milstein, Butcher and Howard (1977) or Gefter, Margulies and Scharff (1977). They have been described and reviewed many times recently (Kennett 1979; Köhler 1979; Fazekas de St Groth and Scheidegger 1980; Goding 1980; Kennett, McKearn and Bechtol 1980; Milstein 1980; Milstein and Lennox 1980; Oi and Herzenberg 1980; Pearson *et al.* 1980; Zola 1980). AFCs for hybridisation are usually taken from the spleen of immunized animals. However, cells from lymph nodes, peripheral blood, lymphatic ducts and stimulated cultures of B cells have also been used. Two or 3 injections of antigen into the donor animal appear to be optimal for hybridoma production (Stähli, Staehelin, Miggiano, Schmidt and Haring 1980). The cells are harvested 3 d to 4 d after the final injection. The type of AFC that fuses is undefined but is possibly a rapidly dividing, immature B cell (reviewed by Goding 1980).

BALB/c mice are normally used as AFC donors because the myeloma cells used for fusion are of BALB/c origin. The hybridomas can then be grown *in vivo* without resorting to immunosuppression of the recipients. Non-syngeneic recipients must be congenitally athymic, treated with anti-lymphocyte serum, irradiated and/or appropriate F₁ hybrids.

For fusion, about 10^7 myeloma cells from log phase growth are mixed with 10^8 spleen cells (see Fig. 2.1). PEG of molecular weights from 1,000 to 6,000 in marginally sub-lethal dosages is almost universally added as the fusogen. The mechanism of hybridisation is poorly understood beyond the fact that plasma membrane cohesion is followed by fusion (Pontecorvo, Riddle and Hales 1977; De Micco, Pautrat, Benkoel, Pouget and Tamalet 1978; Poste and Nicolson 1978; Knutton and Pasternak 1979). The nuclei fuse during the next cell division.

The fusion mixture is incubated in 48 or 96 culture wells in medium that contains hypoxanthine, thymidine and aminopterin (HAT medium; Littlefield 1964) which kills the myeloma cells. These cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT; E.C.2.4.2.8) and are unable to synthesise nucleic acids from hypoxanthine and thymidine salvage (emergency) pathways. The normal (*de novo*) pathways are blocked by aminopterin. Thus, unfused myeloma cells die within 3 days. Spleen cells have a short life span in culture but if they fuse with myeloma cells, the HGPRT gene of the spleen cell allows the hybrid to survive in HAT and the malignant property of the myeloma parent permits unlimited *in vitro* propagation.

1.3.3 Screening for Specific Antibody

Hybrids lose chromosomes in an apparently random manner, particularly at the first division (Davidson 1974; Bernhard 1976; Ringertz and Savage 1976). They stabilize with time but reversion

to HGPRT⁻ or Ig⁻ status often occurs. It is, therefore, best to screen cultures for secretion of specific antibody as soon as possible after fusion, normally when the colony has grown to the point where the culture medium is acidified.

Usually, screening methods are modifications of assays already in use for detecting antibodies to the particular antigen involved. The chosen method depends on the type of antigen (eg. cell surface or soluble), its availability and degree of purity, access to equipment and the length of time taken to assay several hundred samples.

Hybrid cell screening methods have included immunoprecipitation (Coffino and Scharff 1971; Cook and Scharff 1977; Raschke 1978; Springer *et al.* 1978; Clark, Everett, Fitch, Frogner, Jakovcic, Rabinowitz, Warner and Zak 1980; Croce, Linnenbach, Hall, Steplewski and Koprowski 1980b), immunodiffusion (Margulies *et al.* 1976; Laskov, Kimm and Asofsky 1978; Notenboom, Chou, Good, Dubiski, Cinader and Köhler 1980), lytic assays such as haemolysis (Köhler and Milstein 1975; Galfre *et al.* 1977; Pages and Bussard 1978; J. Howard and Corvalan 1979; Pierres, Ju, Waltenbaugh, Dorf, Benacerraf and Germain 1979; Zagury, Phalente, Bernard, Hollande and Buttin 1979; Bankert, Des Soye and Powers 1980), ⁵¹chromium release from nucleated targets (eg. Galfre *et al.* 1977; Pearson, Galfre, Ziegler and Milstein 1977; McKearn, Fitch, Smilek, Sarmiento and Stuart 1979) and, later, trypan blue exclusion (Lemke, Hämmerling, Höhmann and Rajewsky 1978; Trucco, Stocker and

Ceppellini 1978; McMichael, Pilch, Galfre, Mason, Fabre and Milstein 1979). However, binding assays have been most commonly used because they do not require the monoclonal antibody to precipitate antigen or fix complement. Indeed, many MCA do not have these properties (Goding 1980; Milstein 1980; Yelton and Scharff 1980).

It is now recognised that a hybrid cell screening assay should be the most sensitive assay available for the particular antigen (Oi and Herzenberg 1980; Zola 1980). Hence, the binding assays that have been employed for screening are indirect fluorescence on cells (eg. Brodsky, Parham, Barnstable, Crumpton and Bodmer 1979; Ledbetter and Herzenberg 1979; Reinherz, Kung, Goldstein and Schlossman 1979; Brooks, Beckman, Bradley, McNamara, Thomas and Zola 1980; Eisenbarth, Haynes, Schroer and Fauci 1980; Pearson *et al.* 1980) or immobilized soluble antigens (Parks, Bryan, Oi and Herzenberg 1979; Goding 1980; Phillips, Kendal, Webster, Feorino and Reimer 1980), rosetting (eg. Clevinger, Hansburg and Davie 1978; Oi, Jones, Goding, Herzenberg and Herzenberg 1978; Juy, Legrain, Cazenave and Buttin 1979; Kerbel 1980), IHA (Andersson and Melchers 1978; Dipauli and Raschke 1978; Hämmerling *et al.* 1978; Pierres *et al.* 1979), ELISA (Kearney *et al.* 1979; Kelly, Levy and Sikora 1979; Uotila, Engvall and Ruoslahti 1980; Warren, Fanger and Neet 1980; Yarmush, Gates, Weisfogel and Kindt 1980; Kincade, Lee, Sun and Watanable 1981; Ross, Reis and Joh 1981) and RIA (reviewed by Melchers, Potter and Warner 1978; Goding 1980; Eisenbarth 1981).

MCA to cell surface antigens are usually screened with an RIA or fluorescence assay. For soluble antigens, increasing use is being made of fluorescence using immunomicrospheres as the solid phase and mechanised (fluorescence activated cell sorter or cytofluorometer) fluorescent cell sorting (Parks *et al.* 1979; Goding 1980; Rembaum and Dreyer 1980), ELISA and solid phase RIA. The simplest and now most widely applied RIA for antibodies to soluble antigens employs polyvinyl chloride microtitration trays as the solid phase (Koprowski, Gerhard and Croce 1977; Kennett, Denis, Tung and Klinman 1978; Effros, Frankel, Gerhard and Doherty 1979; Parks *et al.* 1979; Pierres *et al.* 1979; Barnstable 1980; Damiani, Frascio, Benatti, Morelli, Zocchi, Fabbi, Bargellesi, Pontremoli and De Flora 1980; Lewicki, Brandwein, Waldman and Murad 1980; Businaro, Butler, Rubenstein and Revoltella 1981). These will probably be the main screening methods in the future, particularly as more laboratories have access to automated measurement of the results.

MCA to parasite surface antigens have been detected by RIA (Mitchell, Cruise, Chapman, Anders and Howard 1979) or fluorescence (Verwaerde, Grzych, Bazin, Capron and Capron 1979; Freeman, Trejdosiewicz and Cross 1980; Pearson *et al.* 1980; Perrin, Ramierez, Er-Hsiang and Lambert 1980; Pinder and Hewett 1980; Rener, Carter, Rosenberg and Miller 1980; Sethi, Endo and Brandis 1980; Yoshida, Nussenzweig, Potocnjak, Nussenzweig and Aikawa 1980; Johnson, McNamara, Neoh, McDonald and Zola 1981; Lyon, Pratt, Travis, Doctor and Olenick 1981) whereas IHA (Johnson *et al.* 1981) and various types of RIA (Craig, Mitchell, Cruise and Rickard 1980; Handman, Goding and Remington 1980;

Hillyer and Pelley 1980; Kim, Taylor, Evans and Asofsky 1980; Mitchell, Cruise, Garcia and Anders 1981) have been used with soluble parasite antigens. For each parasite species, the most sensitive antibody detection method was employed.

1.3.4 Cloning

Once cell culture wells containing antibody of the desired specificity are located, which may be up to 1 in 10 of those that contain Ig (Köhler and Milstein 1976), cloning is necessary. This minimises losses due to chromosome shedding and/or overgrowth by non-secreting clones. Cloning also ensures that each culture is producing only one antibody.

Hybrid cells may be cloned by dilution in 96 well trays, agar or agarose or by micromanipulation of cells into individual wells. Clones are usually grown with a pre-existing culture that acts as a feeder to enhance the survival rate of clones. In order to avoid *in vitro* immune responses if the feeder cells are spleen cells, peritoneal cells or thymocytes, they must be syngeneic to the cloned cells unless the 2 cultures cannot directly contact one another. The resulting cell lines yield 10 to 50 μ g of antibody per ml *in vitro* or 3 to 20 mg/ml if grown as tumours (Schneider and Eisenbarth 1979; Oi and Herzenberg 1980).

Insurance against later reversion to non-secretion or infection or death of cultures, involves storage under liquid nitrogen and periodic recloning.

1.3.5 *Non-murine Monoclonal Antibodies*

The production of interspecific hybrids other than rat x mouse hybrids has been difficult (Schulman and Köhler 1979). Rat MCA are usually desirable as antibodies to non-H2 mouse antigens (Ledbetter and Herzenberg 1979; McKearn *et al.* 1979). Although non-rodent cells fuse with mouse cells, chromosome loss is frequently so rapid that non-murine Ig secretion is usually absent before the first screening test can be performed.

Human x mouse hybrids are notoriously unstable (Kano, Knowles, Koprowski and Milgrom 1972; Croce *et al.* 1973; Schwader and Corvalan 1973; Ringertz and Savage 1976). Several experiments have yielded a small proportion of hybrids that secreted non-specific (Hengartner, Luzzati and Schreier 1978; Koprowski, Gerhard, Wiktor, Martinis, Shander and Croce 1978; Levy and Dilley 1978; Seravalli, Schwab, Pernis and Siniscalco 1978; Smith and Hirschhorn 1978) or specific (eg. Astaldi, Janssen, Lansdorp, Willems, Zeijlemaker and Oosterhof 1980; Croce, Shander, Martinis, Cicurel, D'Ancona and Koprowski 1980a) human Ig but almost all hybrids lose their ability to secrete human Ig after a few months of culture. The chromosomes of the myeloma cell parent are preferentially retained (Seravalli *et al.* 1978; Smith and Hirschhorn 1978; Croce *et al.* 1980a); only 2 human chromosomes persist in these hybrids while they secrete human Ig (Croce *et al.* 1980a). This has caused a number of workers to seek alternative methods of producing human MCA. The procedure originated by Luzzati, Hengartner and Schreier (1977) and Steinitz, Klein, Koskimies and Makela (1977) was to culture human B cells in the presence

of antigen and the transforming agent EBV. Unfortunately, few transformed B cell lines secrete antibody for more than a month in culture.

Spleen cells of frogs (Hengartner *et al.*, 1978) and rabbits (Schulman and Köhler 1979; Notenboom *et al.* 1980; Yarmush *et al.* 1980) have been fused with mouse myeloma cells. Like human x mouse hybrids, these interspecific hybrids either did not secrete non-murine Ig or soon lost this characteristic. Curiously, the only study to include karyotype analyses found no rabbit chromosomes in the rabbit Ig secreting hybrid (Yarmush *et al.* 1980). There have been no reported attempts to fuse B cells of other species with mouse myeloma cells.

The situation with respect to rabbit x mouse hybridomas has recently changed. Unlike previous work that produced rabbit Ig of undefined specificity, Businaro *et al.* (1981) obtained a number of rabbit MCA to mouse nerve growth factor. Their success was attributed to the provision of irradiated rabbit leukocyte feeder cells to the postfusion mixture and screening for specific antibody 4 d after fusion instead of the usual 2 to 3 weeks.

An alternative and preferable means of producing non-murine MCA is to avoid interspecific fusion by using a myeloma of the appropriate species that is suitable for hybridoma production. Rat myelomas - 210 RCY3 Ag1.2.3 (Galfre, Milstein and Wright 1979) and S/194/5 XXO Bul (Trowbridge 1978) - have been available for some time. After many years of effort (cf. Hengartner *et al.* 1978 with Olsson

and Kaplan 1980), human myelomas suitable for fusion have been isolated. They are GM 1500.6TG - A12 (Croce *et al.* 1980b) and U-266AR₁ (Olsson and Kaplan 1980). This is a breakthrough in the application of hybridoma technology to medicine because, theoretically, apart from the risk of small anti-idiotypic responses in patients, anti-antibody responses can now be avoided in human immunization and tumour therapy with MCA. A more immediate boon is the facilitation of producing anti-HLA MCA for tissue typing. This has previously been difficult with xenogeneic MCA (Schroder 1980; Ziegler 1980; Zola 1980).

Rats, mice and humans are the only animals with which it is possible to produce intraspecific hybridomas and only in mouse and human B cells can transformation be induced in order to produce MCA. No myelomas of any kind, from which myelomas suitable for fusion could be selected, have been obtained from other species. Therefore, sources of MCA of these other species are restricted to interspecific fusion and, possibly, spleen fragment cultures (see Section 1.3.1).

Even though most interspecific fusions do not yield MCA of known specificity, such fusions have a purpose apart from that of producing specific antibodies. The resulting MCA, whether antigen-binding or not, are valuable for studying the genetics and immunochemistry of the Ig (Notenboom *et al.* 1980; Yarmush *et al.* 1980).

1.3.6 T Cell Hybridomas

T cell hybridomas are products of T cells and T cell lymphomas. Early attempts to produce functional T cell hybrids failed.

Even now, a satisfactory T cell lymphoma for functional T cell hybridoma production has yet to be discovered. Chromosome shedding is a major problem that has not been overcome. However, there are T cell hybridomas now available that secrete interleukin 2 (Harwell, Skidmore, Marrack and Kappler 1980), allogeneic effector factor (Katz, Bechtold and Altman 1980), T cell growth factor (TCGF; Schrader and Clark-Lewis 1981) and antigen specific or non-specific suppressor factors (see reviews by Melchers *et al.*, 1978; Altman and Katz 1980; Raschke 1980).

Until recently there were no cytotoxic T cell (T_C) hybridomas. Nabholz, Cianfriglia, Acuto, Conzelmann, Haas, Boehmer, McDonald, Pohlitz and Johnson (1980) used various manipulations to overcome this problem. They fused a lymphoma with a TCGF-dependent cytolytic T cell line and isolated T_C hybrids. However, the steps from this procedure to one that would yield T_C hybrids from fusing a lymphoma with sensitised, untransformed T cells are obscure. Thus, cloning activated T cells is still more successful than hybridisation for producing monoclonal, functional T cells (Srendi, Tse, Chen and Schwartz 1981).

1.3.7 Applications of Monoclonal Antibodies

Hybridoma technology has been applied to a vast number of problems; numerous reviews deal with the most common applications. As well as general accounts (Melchers *et al.* 1978; Blann 1979; Scharff 1979; Kennett *et al.* 1980; Milstein 1980; Raschke 1980; Staines and Lew 1980; Eisenbarth 1981) there are discussions of hybridomas and transplantation antigens (Brodsky *et al.* 1979; Gotze

and Vollmers 1979; J. Howard, Butcher, Galfre, Milstein and Milstein 1979; Lemke, Hämmerling and Hämmerling 1979; McKearn *et al.* 1979; McMaster and Williams 1979; Zola 1980), leukocyte differentiation antigens (Ledbetter, Rouse, Micklem and Herzenberg 1980; Milstein and Lennox 1980; Reinherz, Moretta, Roper, Breard, Mingari, Cooper and Schlossman 1980; Zola 1980; Janossy, Tidman, Papageorgiou, Kung and Goldstein 1981), malaria (Cox 1980), the neurosciences (Cuello, Galfre and Milstein 1980; Inglis 1980), idiotypes (Sharon, Morrison and Kabat 1979; Kelsoe, Reth and Rajewsky 1980), genetics (Hansburg, Clevinger, Perlmutter, Griffith, Briles and Davie 1979; Maki, Kearney, Paige and Tonegawa 1980; Yelton and Scharff 1980) and medicine (Lajtha 1980; Schroder 1980; Staines and Lew 1980; Ziegler 1980).

Melchers *et al.* (1978), Schulman and Köhler (1979) and Raschke (1980) have listed a number of hybridomas. The most common are to cell (mostly leukocyte) surface antigens because preparation of conventional antisera to these antigens is extremely tedious, and it is easier to produce MCA to them than to soluble antigens. The former MCA have been used either in tissue typing or studying leukocyte subsets or Ia antigens (Raschke 1980; Reinherz *et al.* 1980; Zola 1980; Eisenbarth 1981; Janossy *et al.* 1981). Human T cell subsets have attracted the greatest attention.

Viral serology has been clarified and the analysis of antigenic change in influenza facilitated with hybridomas (eg. Koprowski *et al.* 1977; Effros *et al.* 1979; Laver, Air, Webster, Gerhard, Ward and Dopheide 1979; Phillips *et al.* 1980).

The time consuming, complex screening procedures involved in attempting to obtain tumour-specific antibodies are not avoided by using hybridoma technology (eg. Levy, Dilley and Lampson 1978; Stern, Willison, Lennox, Galfre, Milstein, Secher and Ziegler 1978; Brown, Tamerius and Hellstrom 1979; Scharff 1979; Steplewski, Herlyn, Herlyn, Clark and Koprowski 1979; Youle and Neville 1980) but the resulting MCA do not require absorption and are standard, limitless preparations. Research on antigens peculiar to embryos has benefited similarly (Solter and Knowles 1978). In endocrinology, there are MCA to hormone receptors (Greene, Nolan, Ergler and Jensen 1980; Greene, Fitch and Jensen 1980) and MCA have been made which, *in vitro* on adipocytes, mimic the action of insulin (Beachy and Czeh 1980). Hybridoma technology has also been applied to entomology (Brower, Smith and Wilcox 1980; G. Howard, Abmayr, Shinefeld, Sato and Elgin 1981), botany (Liedgens, Gruetzmänn and Schneider 1980; Rathjen, Gorrell and Chin 1981) and autoimmune diseases (Pages and Bussard 1978; Andrzejewski, Stollar, Lalor and Schwartz 1980; De Heer, Pages and Bussard 1980; Eilat, Asofsky and Laskov 1980). Myosin (Clark *et al.* 1980), Ig isotypes (Bordenave, Pages, Stoltz and Bussard, 1979; Galfre *et al.* 1979; Pinder, Musoke, Morrison and Roelants 1980; Zitron and Clevinger 1980) and allotypes (Dipauli and Raschke 1978; Goding, Oi, Jones, Herzenberg and Herzenberg 1979; Parks *et al.* 1979), mouse Fc receptors for IgG_{2a} (Pieris, Gordon, Unkeless and Porterfield 1981) and IgG_{2b} (Mellman and Unkeless 1980), osteoclast activating factor (Luben, Mohler and Nedwin 1979), human interferon (Secher and Burke 1980), complement component C3 (Lachmann, Olroyd, Milstein and Wright 1980) and coagulation factor (Katzmann, Nesheim, Hibbard and Mann 1981), hepatitis B

surface antigen (Shih, Cote, Dapolito and Gerin 1980), sperm (Bechtol, Brown and Kennett 1979; Shigeta, Watanabe, Maruyama, Koyama and Isojima 1980; Myles, Primakoff and Bellve 1981), Con A binding protein (Starling, Simrell, Klein and Noonan 1979), human alphafetoprotein (Uotila *et al.* 1980), peroxidase (Zagury *et al.* 1979) and other enzymes (Damiani *et al.* 1980; Lewicki *et al.* 1980; Ross *et al.* 1981) are further examples of targets of MCA produced so far.

MCA to leukocyte subsets have been used to purify or lyse those subsets (Ng, Indiveri, Pellegrino, Molinaro, Quaranta and Ferrone 1980; Raulet, Gottlieb and Bevan 1980; Saxena, Adler and Nordin 1980). The specificity of the poison ricin can be enhanced by linking it to MCA directed against the target cell type (Youle and Neville 1980). This type of approach may be applied to tumour chemotherapy and may include using human anti-idiotypic MCA to treat multiple myeloma (Ziegler 1980). Another medical application of MCA is in the diagnosis of cancer and foetal malformations (Uotila, Ruoslahti and Engvall 1981).

MCA can be used in recombinant DNA technology by incorporating them into the screening tests for cDNA clones (Kemp and Cowman 1981).

Some researchers have localised the antigens of internal organs to which their MCA bind using fluorescent antibody or peroxidase methods (Cuello, Galfre and Milstein 1979; Barnstable 1980; Izant and McIntosh 1980; G. Howard *et al.* 1981; Ross *et al.* 1981; Zipser

and McKay 1981). Many analyses of MCA have included isolation of the corresponding antigens by immunoprecipitation (eg. Starling *et al.* 1979; Clark *et al.* 1980; Croce *et al.* 1980b; Izant and McIntosh 1980; Liedgens *et al.* 1980; Coffman and Weissman 1981; Le Clair, Van Agthoven and Terhorst 1981; Ross *et al.* 1981) or affinity chromatography (eg. McMaster and Williams 1979; Parham 1979; Damiani *et al.* 1980; Fraser and Venter 1980; Mellman and Unkeless 1980; Secher and Burke 1980; Katzman *et al.* 1981).

1.3.8 Applications to Parasitology

Most of the anti-parasite MCA have been to antigens of the protozoans *Plasmodium falciparum* (Perrin *et al.* 1980), *P. gallinaceum* (Rener *et al.* 1980), *P. knowlesi* (Epstein, Miller, Kaushel, Udeinya, Rener, Howard, Asofsky, Aikawa and Hess 1981), *P. berghei* (Potocnjak, Yoshida, Nussenzweig and Nussenzweig 1980; Yoshida *et al.* 1980; Perrin, Ramirez, Lambert and Miescher 1981), *P. yoelii* (Freeman *et al.* 1980; Kim *et al.* 1980; Bryz-Gornia, Long, Daly, Klinger and Weidanz 1981), trypanosomes (Pearson *et al.* 1980; Pearson, Kar, McGuire and Lundin 1981; Lindsley, Janecek and Esser 1981), *Theileria parva* (Pinder and Hewett 1980) and *Toxoplasma gondii* (Araujo, Handman and Remington 1980; Handman and Remington 1980; Handman *et al.* 1980; Sethi *et al.* 1980; Johnson *et al.* 1981). The cestodes *M. corti* (Mitchell *et al.* 1979), *E. granulosus*, *T. hydatigena* and *T. ovis* (Craig *et al.* 1980) and the blood flukes *S. mansoni* (Verwaerde *et al.* 1979; Hillyer and Pelley 1980) and *S. japonicum* (Mitchell *et al.* 1981) have also been hybridoma targets.

Obtaining species specific MCA appears to be subject to chance rather than being guaranteed. Considering the high degree of antigen sharing by parasites (Capron *et al.* 1968) this would be expected. Even so, species specific (Mitchell *et al.* 1979, 1981; Kim *et al.* 1980; Pearson *et al.* 1980; Perrin *et al.* 1980) as well as genus specific (Craig *et al.* 1980; Hillyer and Pelley 1980; Perrin *et al.* 1981) MCA have been produced.

Anti-parasite MCA have been used to develop immunodiagnostic tests (Mitchell *et al.* 1979, 1981; Araujo *et al.* 1980; Hillyer and Pelley 1980; Kim *et al.* 1980) and in attempts to passively protect hosts against infection (Freeman *et al.* 1980; Rener *et al.* 1980) or kill parasites *in vitro* (Verwaerde *et al.* 1979; Handman and Remington 1980; Potocnjak *et al.* 1980; Yoshida *et al.* 1980; Epstein *et al.* 1981). One such study of particular interest is that by Verwaerde *et al.* (1979) who produced rat MCA that exhibited either complement or eosinophil dependent cytotoxicity against schistosomes. Radiolabelled antigens of *P. falciparum* (Perrin *et al.* 1980, 1981), *P. berghei* (Yoshida *et al.* 1980), *P. yoelii* (Bryz *et al.* 1981), *Trypanosoma rhodesiense* (Lyon *et al.* 1981) and *T. gondii* (Handman *et al.* 1980) have been isolated by immunoprecipitation. Sepharose columns have been employed in purifying cestode (Craig, Hocking, Mitchell and Rickard 1981), trypanosome (Pearson and Anderson 1980) and *P. knowlesi* (Epstein *et al.* 1980) antigens. Successful antigen isolation is demonstrated by SDS polyacrylamide gel electrophoresis (PAGE). Sites of MCA binding to parasites have not been determined except where immunofluorescence on whole parasites has

been used as a screening method. This screening method has been used on schistosomula (Verwaerde *et al.* 1979) and with almost all MCA to protozoans (see Section 1.3.3).

1.4 RESEARCH PROJECT

Although such procedures as stripping off the tegument (Hillyer 1980), raising antisera to immunoprecipitated antigens (Howell *et al.* 1977; Hillyer and Cervoni 1978; Yoshihara *et al.* 1979), affinity chromatography (Hillyer and Cervoni 1978) and physiochemical fractionation (Korach and Benex 1966a; Tailliez and Korach 1971a; Behm and Bryant 1980; Simpkin *et al.* 1980) are adequate for the task of analysing fluke antigens, none are entirely satisfactory in that they are applicable to few antigens and do not produce standardised, monospecific reagents. Therefore, there is a need to explore novel methodologies. One such approach is investigated here.

The project aim was to examine the application of hybridoma technology to the study of fascioliasis from the viewpoints of antigenic analysis, immunodiagnosis and vaccination. It was envisaged that spleen cells from (1) mice immunized with a crude fluke extract would be useful for adapting the necessary techniques, including screening assays and antigen localisation and isolation to the study of flukes, (2) infected sheep could be used for the production of an immunodiagnostic reagent and (3) infected rats and rats immunized with immunoprecipitated fluke excretory/secretory antigens could parent hybridomas that could then be employed in passive immunization experiments followed by isolation and localisation of functional antigens.

Chapter 2

PRODUCTION OF SOMATIC CELL HYBRIDS

2.1 INTRODUCTION

As indicated in Section 1.4, it was thought that application of the techniques of B cell hybridisation described by Köhler and Milstein (1975, 1976) and Galfre *et al.* (1977) could overcome the limitations of physicochemical methods for studying individual antigens of complex mixtures.

The production of hybridomas has 3 prerequisites: (1) antigenic stimulation of animals; (2) fusion between antibody-forming and myeloma cells and (3) identification of hybrid cells that secrete antibody of the desired specificity.

Both infected and immunized animals were used as sources of AFCs. It was considered that infected animals, although they generally exhibit lower antibody titres than animals which are artificially stimulated with antigens (see Section 1.2.2.2) and are accordingly a poorer source of AFCs, would enable MCA of greater value for immuno-diagnosis to be derived. In addition, infected rats were expected to be a source of AFCs from which MCA to functional antigens could be derived because this host can resist challenge infections and this resistance can be passively transferred with serum (see Sections 1.2 and 1.2.2.3).

Immunization of animals increases the proportion of anti-*F. hepatica* AFCs in spleens and could be expected to increase the range of MCA that are ultimately derived. Therefore, rats immunized with

immunoprecipitated antigens that include one or more functional antigens (Howell 1979; Howell and Sandeman 1979) and mice immunized with a crude fluke extract were used. It should be noted that antigens effective as vaccines may be in a subset that does not elicit a response during infection (Mitchell 1979b). Some mice were immunized with fluke antigens coupled to RBC because (1) it has been shown that higher titres of antibodies to soluble antigens can be obtained by immunization with antigen-coated syngeneic RBC than with antigen alone (Ramshaw and Parish 1977) and (2) binding of macromolecules to RBC induced by the agent used here, chromic chloride, is selective (Parish and McKenzie 1978). Since antigen-coated RBC were to be used in the screening method (IHA) it was reasoned that the probability of detecting mouse MCA would be increased by adopting this immunization method.

Although non-rodent MCA are difficult to produce (see Section 1.3.5), infected sheep were employed as a source of anti-*F. hepatica* AFCs because infection of this host is of economic importance. Moreover, it was thought that sheep may respond to some antigens to which rats and mice do not; a view that has since been substantiated (Lehner and Sewell 1980).

Following fusion, the main objective was to detect hybrids secreting MCA to *F. hepatica* antigens. Thus, there was a need for suitable screening procedures for this purpose. There are many established methods for detecting anti-fluke antibodies (see Section 1.2.2.1). Immunodiffusion (ID) is the simplest and most widely used but the sensitivities of IEP, CEP and IHA are much greater. Hillyer and

and Allain (1979) showed that the last-named methods are of equal sensitivity. However, that of IHA can be greatly enhanced if antigens are chemically attached to SRBC using CrCl_3 rather than passively adsorbed (Ling, Stephens, Bratt and Dhaliwal 1979). The CrCl_3 coupling method has been optimized by Parish and Haywood (1974), Goding (1976a) and Parish and McKenzie (1978). SRBC have been coated with *T. taeniaeformis* antigens in this way (Kwa and Liew 1978), showing that the method is applicable to helminth parasite antigens. Another consideration was that SRBC treated with CrCl_3 remain susceptible to lysis mediated by antibody and complement (Goding 1976a). This meant that hybrid cells could be screened by growing them in agar then overlaying them with fluke antigen-coated SRBC and complement (haemolytic plaque assay) and placing drops of spent culture medium (supernatants) on agarose gels containing fluke antigen-coated SRBC and complement (haemolytic spot assay) as well as IHA.

Immunoprecipitation over clones growing in agar, haemolytic plaque, ID and IHA methods had, at the initial stages of this project (1978), been used successfully to identify hybridomas of other specificities (see Section 1.3.3). Therefore, these were the first methods tested for their suitability in the present study.

The production of hybrid cells and initial attempts to detect those which secreted anti-fluke antibody by the above methods are described in this Chapter.

2.2 MATERIALS AND METHODS

2.2.1 Antigens

Flukes were recovered from the bile ducts of sheep slaughtered at Goulburn Abattoir, NSW, and incubated in Hedon-Fleig saline (Cornish and Bryant 1976) at 37°C for 5 h to allow them to regurgitate their caecal contents. Any flukes which still contained caecal material after this time were discarded; the remainder were homogenised in 0.9% NaCl (5 ml/g wet weight) in a Kontes tissue grinder on ice. Immunization of various species of mammals used antigen in this form (FHH). FHH was clarified by centrifugation at 1000 g at 4°C for 20', then at 40 000 g for 30'. The supernatant, designated FHS, was adjusted to 1 mg protein/ml and stored at -20°C. Protein determinations were carried out by the Lowry method (Lowry *et al.* 1951) using BSA as the standard.

The incubation products of excysted metacercariae (MFIP) were collected after 1 week *in vitro* as described by Howell and Sandeman (1979). Those of adult flukes (FIP) were collected between 5 h and 2 d after removal from the bile ducts of sheep and incubation in Hedon-Fleig saline. MFIP and FIP were concentrated to 1 mg/ml by dialysis against 20% PEG (Aquacide III, Calbiochem) in 0.9% NaCl.

2.2.2 Immunizations and Infections

Animals were sensitised to fluke antigens prior to harvesting spleens and sera for (1) fusion with mouse myeloma cells, (2) developing haemolytic and IHA screening assays and (3) measuring serum anti-fluke titres of spleen cell donors.

Merino cross lambs were administered metacercariae *per os* as described by Sandeman and Howell (1980, 1981a). A lamb given 200 metacercariae was necropsied 16 weeks after infection. It was found to be infected with 71 adult flukes. A spleen cell suspension was prepared from this animal (see Section 2.2.4). At this time a second lamb, initially given 100 metacercariae, was reinfected with 100 metacercariae. At necropsy 20 weeks later, a spleen cell suspension was prepared from this animal and 82 flukes were recovered from its bile ducts.

Sera from 3 infected sheep were pooled 9 weeks after infection with 100 metacercariae.

Three male Wistar rats were immunized with immunoprecipitated adult (R1) or metacercarial (R2 and 3) antigens. The immunoprecipitates were prepared by aseptic incubation of parasites in immune rat sera as described by Howell *et al.* (1977). Each rat was injected in the hind foot pads with 80 to 100 µg of precipitate in Freund's complete adjuvant (FCA) twice, 4 weeks apart and killed 7 d later for the preparation of sera (RA1 and RA2) and spleen cells.

Male (R5 and 6) and female (R4, 7 and 8) 6 weeks-old Wistar rats were given 10 (R5 and 6) and 20 (R4, 7 and 8) metacercariae of *F. hepatica* by stomach tube. Spleens were removed from 2 rats 25 d (R7) and 35 d (R8) later. These rats were infected with 13 and 17 immature worms respectively. Challenge infections of 30 metacercariae were given to the remaining rats 8 weeks after the

first. Rat 5 was found to be infected with 3 adult flukes when its serum (IR5) and spleen were harvested 15 d later. Third infections, 16 weeks after the first, were of 30 metacercariae. Rats 4 and 6, which were killed 20 and 32 d later, were infected with 4 and 11 adult flukes respectively.

An antiserum to sheep serum was produced by giving each of 2 Wistar female rats an intraperitoneal injection of 2 ml of an emulsion of equal parts of normal sheep serum and FCA. Two further 1 ml injections of equal parts of serum and Freund's incomplete adjuvant (FiCA) were given at 3 week intervals. Serum was collected 10 d after the last injection.

Ten mice were immunized with fluke antigens; 4 BALB/c males were given a series of 1 ml injections of 5 mg FHH emulsified with FCA in equal proportions (M1, 2, 3 and 4) and 6 males (2 BALB/c and 4 CBA) were given injections of RBC coated with FHS (M5, 6, 7, 8, 9 and 10) as described in Section 2.2.3. CBA mice were used when BALB/c mice were not available. All injections were administered intraperitoneally, 3 to 4 weeks apart. The sera of 3 BALB/c mice given 3 injections of FHH were collected 4 d (MA1) and 10 d (MA3 and MA4) after the final injection. The other BALB/c antiserum to FHH (MA2) was collected 4 d after a second injection. One BALB/c mouse was injected with 10^9 then 2×10^8 FHS-coated BALB/c RBC and 2×10^9 coated SRBC; serum was prepared 8 d later (MA5). Antiserum MA6 was collected 8 d after a third injection of 10^9 coated SRBC.

The CBA mice were injected twice with 7×10^8 coated CBA MRBC. Mice 7 and 8 were killed 4 d after the second injection but mice 9 and 10 were given an additional injection of 10^9 coated SRBC. Serum MA9 and spleens from both mice were harvested 3 d later. Each batch of RBC was washed after the coating procedure to remove FCS and the specified numbers of RBC suspended in 0.4 ml PBS per injection.

In addition, sera from 10, 15 weeks-old CBA mice were pooled 18 d after oral infection with 5 metacercariae of *F. hepatica*.

A female New Zealand white rabbit was injected subcutaneously and intramuscularly with 64 mg FHH in 1 ml PBS emulsified with 1 ml FCA. A further 3 subcutaneous injections at monthly intervals contained a total of 129 mg FHH emulsified with FiCA. The animal was bled from an ear vein 10 d after the final injection. Two female New Zealand white rabbits were immunized by the same protocol with 1 ml aliquots of normal rat or sheep serum emulsified with equal volumes of FCA or FiCA as appropriate.

2.2.3 Coupling Antigens to Red Blood Cells

Blood from sheep and mice was collected in heparinised tubes. RBC were washed, stored in 0.9% NaCl and coupled to FHS 4 to 20 d later according to the methods of Parish and McKenzie (1978). Stock 1% solutions of CrCl_3 (BDH) in 0.9% NaCl were held at pH 4.5 and diluted to 0.1% 1 to 6 months before use. Routinely, 2.5 ml

10% RBC in 0.9% NaCl, 200 μ l 0.45 μ m Millipore filter-sterilized FHS at 1 mg/ml and 150 μ l 0.1% CrCl_3 in 0.9% NaCl at pH 5 were used in the coupling reaction. Vigorous mixing immediately upon addition to CrCl_3 is essential. Titrations that determined optimal amounts of CrCl_3 and FHS for the full scale reaction used 0.5 ml RBC. Attempts to couple MFIP and FIP to SRBC were unsuccessful.

2.2.4 *Cell Fusion*

The procedure for producing hybridomas is summarised in Fig. 2.1. Spleen cell sources for cell hybridizations are described above. The animals used were the 2 sheep, rats 1, 2, 3, 6, 7 and 8 and mice 1, 2, 7, 8, 9 and 10. The spleens of mice 7 and 8 and 9 and 10 were pooled for mouse x mouse fusions 7/8 and 9/10.

Spleen cell suspensions were prepared aseptically by rubbing spleens through a mesh spoon dipped in cold 2% FCS (Flow Laboratories) in pH 7.4 phosphate buffered saline (PBS). After centrifugation at 600 g for 7' the cells were suspended in 7 ml Dulbeccos modified Eagles medium (DMM) or RPMI 1640 (Flow) and held on ice for 5' to enable clumped cells to settle out. The cells remaining in suspension were washed again in DMM and viable cells counted by trypan blue exclusion.

All RPMI 1640 and DMM contained 20 mM N-2-hydroxy-ethylpiperazine-N'-2-ethanesulfonic acid (HEPES; Ultrol, Calbiochem) as buffer.

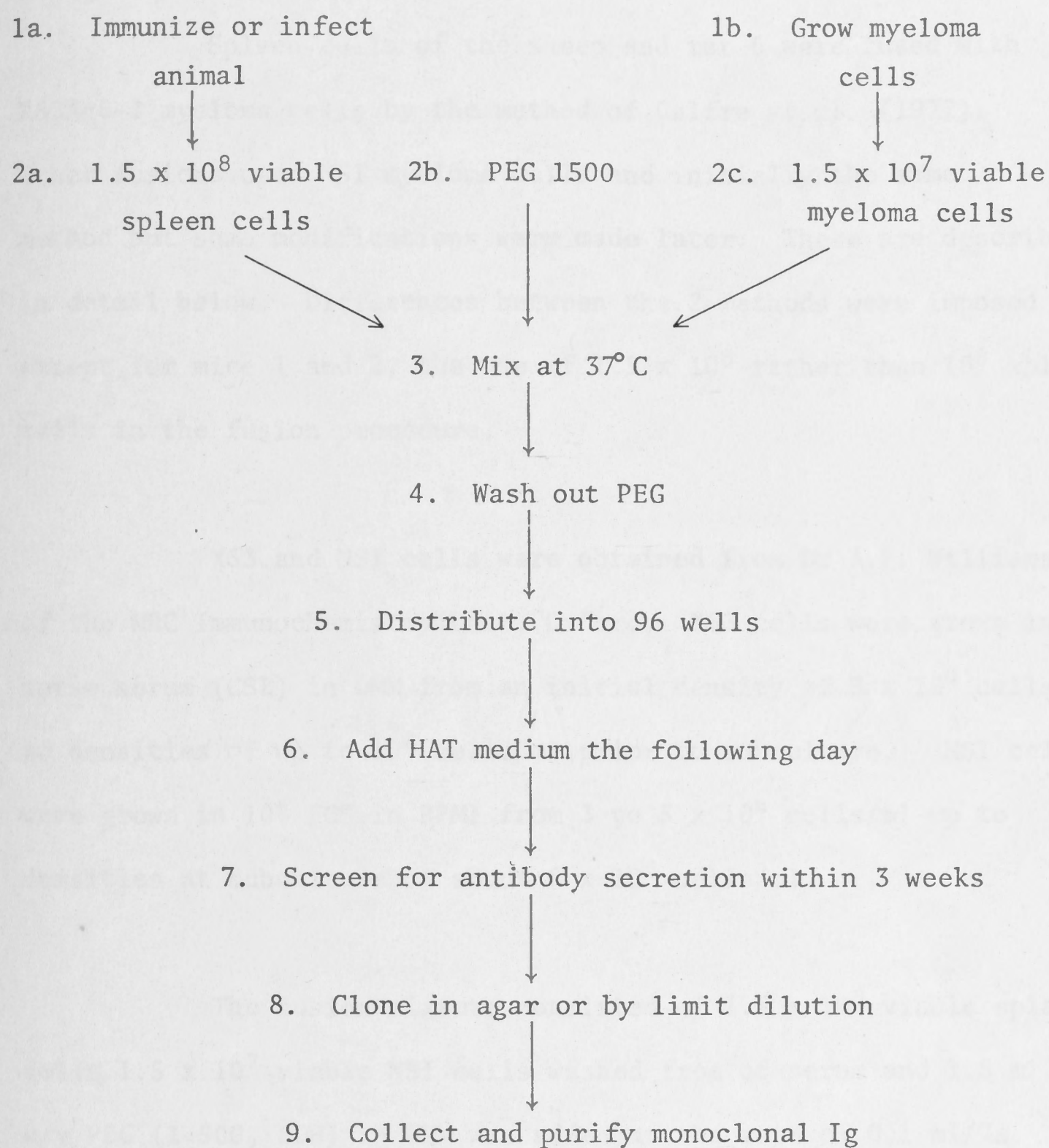


Fig. 2.1. Flow chart of the procedure used to produce hybridomas.

Spleen cells of the sheep and rat 6 were fused with X63Ag8-1 myeloma cells by the method of Galfre *et al.*, (1977). Other fusions used NSI myeloma cells and initially the same method but some modifications were made later. These are described in detail below. Differences between the 2 methods were imposed by, except for mice 1 and 2, the use of 1.5×10^8 rather than 10^8 spleen cells in the fusion procedure.

X63 and NSI cells were obtained from Dr A.F. Williams of the MRC Immunochemistry Unit, Oxford. X63 cells were grown in 10% horse serum (CSL) in DMM from an initial density of 5×10^4 cells/ml to densities of up to 10^6 cells/ml prior to subculture. NSI cells were grown in 10% FCS in RPMI from 3 to 5×10^4 cells/ml up to densities at subculture of about 6×10^5 cells/ml.

The fusion mixture consisted of 1.5×10^8 viable spleen cells, 1.5×10^7 viable NSI cells washed free of serum and 1.5 ml 50% w/v PEG (1 500, BDH) in DMM was added at the rate of 0.1 ml/7s. All solutions used after this step were at 37°C. After agitating for a further 1' 10 ml of DMM was added over 5'. Centrifugation at 600 g for 5' was followed by resuspension of the cells in 50 ml of 20% FCS in RPMI and transferring 1 ml aliquots of the mixture to each well of 24 well Linbro culture trays and incubating at 37°C. For mouse x mouse fusions 7/8 and 9/10 the final volume was 100 ml rather than 50 ml.

The following day 1 ml double strength HAT medium (Littlefield 1964) was added to each well. HAT medium (normal strength) was made by adding 1 ml hypoxanthine (Sigma) at 176 mg/l, 100 μ l aminopterin (grade II, Sigma) at 775 mg/l and 1 ml thymidine (Sigma) at 2.72 g/l to 100 ml 10% FCS in RPMI or DMM. The culture medium in each well was replaced with HAT medium on days 5 and 9 postfusion. On days 12 and 15 postfusion the culture medium was replaced with HT medium, which is identical to HAT but lacks aminopterin. Subsequent medium changes were made with 10% FCS in RPMI at intervals determined by cell growth rates. The cultures were covered with PSM sealers (Flow Cat. No. 76-412-05) to reduce the likelihood of fungal infections. Provision of a 5% CO₂ atmosphere was essential for cell growth in agar and culture trays despite buffering by HEPES.

2.2.5 *Cloning Cells in Agar*

Sheep x mouse and rat x mouse hybrid cells were cloned from 2 ml cultures in 24 well Linbro trays into Petri dishes (30 mm, Falcon) containing a substrate of 3 ml 0.6% agar (Ionagar, Oxoid) in 20% FCS in DMM. This was formed by mixing 0.8 ml FCS, 1.5 ml double strength DMM and 1.5 ml 1.3% aqueous agar per Petri dish. The inoculum of cells was in 0.9 ml 0.4% agar; made by mixing 0.6 ml of base layer agar held at 46°C to 0.3 ml of the cell culture at 37°C. Each Petri dish received from 1 000 to 10 000 cells. Petri dishes were left for 7 d at 37°C in a sealed, humidified chamber gassed with 10% CO₂ in air before examining for clonal growth.

2.2.6 *Screening Clones Grown in Agar*

Two screening methods for the detection of clones of the desired specificity involved overlaying clones growing in agar with appropriate reagents. The first type of overlay contained fluke antigen FHS or an antiserum to sheep or rat Ig (immunoprecipitation method) and the second contained FHS-coated SRBC and complement (haemolytic plaque method).

Immunoprecipitation

The rationale for this test is that the precipitation of antigen-antibody complexes in the agar would reveal the presence of positive clones. The overlay consisted of 0.8 ml per Petri dish of a mixture containing 0.25 ml aqueous FHS at 50 mg/ml, 0.5 ml double strength DMM and 0.25 ml 1.3% aqueous agar. For detecting clones secreting rat or sheep Ig, FHS was replaced by either rabbit anti-sheep serum, rabbit anti-rat serum or fluoresceinated rabbit IgG anti-rat Ig (Wellcome) as appropriate. Control overlays contained normal rabbit serum.

Haemolytic Plaque Assay

After at least one week of culture, clones growing in agar were overlaid with FHS-coated SRBC in 0.9 ml agarose in order to detect the secretion of specific antibody. Agarose was used instead of agar as the latter is anticomplementary. The overlay consisted of 0.4 ml 1.8% aqueous agarose, 0.4 ml double strength DMM, 0.1 ml FCS, 0.1 ml rabbit IgG anti-sheep IgG (Wellcome) or rabbit anti-rat serum diluted 1 in 50 and 0.1 ml 20% FHS-coated

SRBC. Each Petri dish was incubated at 37°C under 5% CO₂ in air for 30' before 0.4 ml guinea pig complement diluted 1 in 10 was added.

The dishes were examined for plaques after incubating them a further 1 to 4 h.

2.2.7 Other Antibody Assays

A Cunningham plaque assay was developed in order to determine the suitability of a haemolytic plaque assay for screening hybrid cells. A haemolytic spot assay, IHA and ID were employed in testing for the presence of anti-fluke antibodies in sera and hybrid cell supernatants. A fluorescent antibody test was used to look for sheep Ig on hybrid cells.

Cunningham Plaque Assay

Cunningham chambers were filled with an assay mixture of 5×10^6 viable rat spleen cells in 100 μ l 2% FCS in PBS, 25 μ l 20% FHS-coated SRBC, 25 μ l guinea pig complement (Commonwealth Serum Laboratories) diluted 1 in 4 and 25 μ l rabbit IgG anti-rat IgG (Wellcome) diluted 1 in 40 for developing IgG plaques. All reagents were in PBS. Chambers were sealed with a mixture of petroleum jelly and paraffin wax in equal proportions and incubated at 37°C for 1 h. Control procedures included uncoated SRBC or normal rabbit serum or omitted complement.

Haemolytic Spot Assay

The haemolytic spot assay used 30 mm Petri dishes containing a 3 ml substrate of 0.9% agarose in DMM and a 0.9 ml overlay of a solution of 0.4 ml 1.8% aqueous agarose, 0.4 ml double strength DMM, 0.1 ml rabbit anti-rat serum diluted 1 in 50, 0.1 ml guinea pig complement and 0.1 ml 25% FHS-coated SRBC. The diluent was DMM. After the agarose solidified, diluted sera were tested by placing 25 μ l samples on the agarose and incubating at 37°C for 1 h.

Immunodiffusion

Immunodiffusion was carried out in 60 mm Petri dishes of 1% ionagar (Oxoid) containing merthiolate at 1 in 10 000. The well volume was 150 μ l. Reagents were reacted for 3 d before washing in 0.9% NaCl then distilled water.

Production of the rabbit anti-sheep serum used in some of the ID tests is described in Section 2.2.2. The sheep IgG that was used was purified by Dr R.M. Sandeman by sephadex G200 gel filtration and sephadex DEAE A50 ion exchange chromatography as described by Sandeman and Howell (1981c).

Indirect Haemagglutination

The method of coating SRBC with FHS antigens is described above. IHA tests were carried out in V-bottom microtitration trays (Linbro) using 2% SRBC in 1% FCS in PBS and PBS as the diluent. All batches of FHS-coated SRBC were standardised against the rabbit anti-FHH serum.

Fluorescent Antibody Test

Sheep x mouse hybrid cells and X63 cells were tested for the presence of sheep Ig on their cell membranes. Cells were washed by centrifugation at 600 g for 5' in 1% FCS in PBS. 100 μ l packed cells were treated with 10 μ l normal rabbit serum or fluoresceinated rabbit IgG anti-sheep IgG (Wellcome) diluted 1 in 10 for 40' at room temperature. After washing, cells were examined using a Leitz Ultraviolet photomicroscope with a BG12 exciter filter.

2.2.8 Cryopreservation of Cells

Cells were frozen in 1 ml 10% dimethyl sulphoxide (DMSO), 10% FCS in DMM or RPMI in 3 ml vials. Each vial contained 1 to 3×10^6 cells. The cells were cooled at about 1°C/min by standing the vials on dry ice in a thermos flask for 30 to 60' at room temperature then 2 to 4 h at -20°C before transferring them to a liquid nitrogen freezer.

Cells were thawed rapidly by transferring vials from liquid N₂ to a 37°C water bath. Cells were then washed once in 10 ml DMM at 37°C, cultured at 2×10^5 /ml and observed daily.

2.2.9 Karyotype Analysis of Hybrid Cells

Chromosomes were enumerated by the following method. To 5 ml cell cultures at densities of about 2×10^5 cells/ml was added 50 μ l 0.01% colchicine (BDH). After 6 to 8 h at 37°C cells were centrifuged at 200 g for 5' then maintained at 37°C in 2 ml fresh, warm 0.5% acetic acid - methanol fixative (in a 1:3 ratio)

was added. Fixation was completed by treatment with 5 ml fixative for 10' then 2 washes in fixative. The chromosomes were heat-spread in burning ethanol and stained with haematoxylin.

2.3 RESULTS

2.3.1 *Sheep x Mouse Hybrids*

Sheep spleen cells, X63 cells and the fusion mixture of these cells were maintained in HAT medium. Within 1 week 5 cell types could be distinguished in the fusion mixture and 4 in the spleen cell cultures. The X63 cell cultures contained no living cells.

Fibroblast-like cells proliferated in most wells of the spleen cell and fusion mixture cultures. About half of these cultures contained colonies of spherical cells, about the size of small lymphocytes, that expanded slowly then receded and disappeared 2 to 5 weeks later. Also present in most wells were as many as 100 spherical cells; these were larger than X63 cells and did not appear to proliferate. The last 2 cell types were almost always accompanied by fibroblastic cells. The latter underwent cycles of proliferation and monolayered in the wells, then receded. They were eventually overgrown by hybrid cells or epithelial-type cells. Epithelial-like cells, first observed several weeks postfusion, tended to dominate after several months those wells in which hybrid cells did not appear.

One cell type was unique to cultures of fusion products. The cells were morphologically similar to X63 cells, grew in clusters and were presumed to be hybrid cells. After the

first fusion this cell type proliferated vigorously to the point where most colonies acidified the medium overnight. Other colonies of hybrids either ceased to grow or died. Twelve cultures were stored under liquid N₂. From the second fusion some cells presumed to be hybrids exhibited limited growth and most died. Others became contaminated with fungus. This proved to be a major problem in 24 well trays with loose fitting lids but was overcome by using sealers.

Ten-fold concentrated supernatants from the hybrid cultures were run against concentrations of FHS from 5 to 50 mg/ml in ID. No precipitin lines were obtained, indicating that if anti-fluke antibody was present it was not detectable by this method. A rabbit anti-sheep serum produced several precipitin lines when reacted against all hybrid and X63 supernatants in ID, presumably due to crossreactivity with the FCS in the culture medium. However, one supernatant formed an additional precipitin line in 1 of 3 replicates. The presence of sheep Ig in this hybrid culture was confirmed by reacting the supernatant against rabbit IgG anti-sheep IgG. One of the resulting precipitin lines formed a line of identity with that formed against purified sheep IgG (see Fig. 2.2).

Karyotypic analysis 2 months postfusion of the hybrid cell culture shown to produce sheep Ig indicated that the cells contained between 52 and 67 chromosomes. Following 8 months storage under liquid N₂ and a further month's growth *in vitro*, less than 48 chromosomes (mean 44) were present. Fluorescent antibody tests on

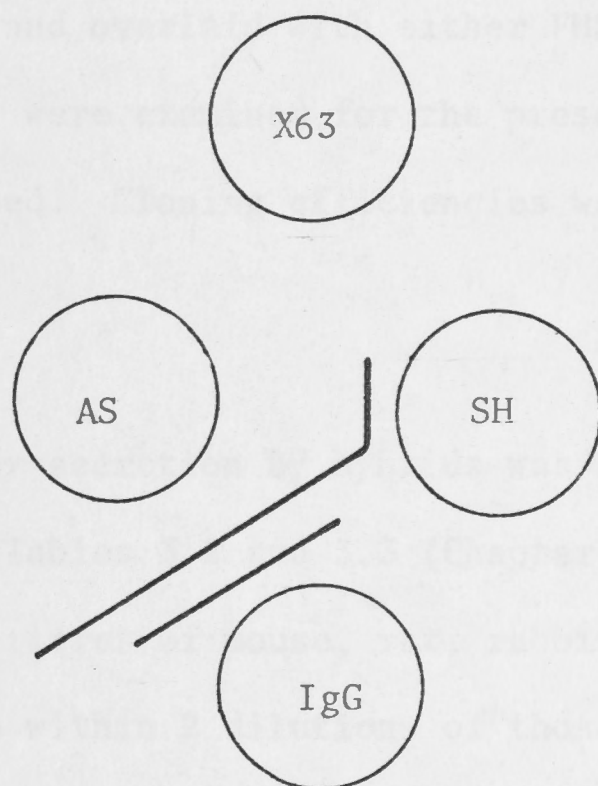


Fig. 2.2. Diagram of immunodiffusion test in which rabbit IgG anti-sheep IgG (AS) was reacted against a sheep x mouse hybrid (SH) and X63 myeloma cell supernatants and purified sheep IgG.

these cells and 4 other hybrids failed to reveal the presence of sheep Ig.

As a further test for antibody secretion 4 cultures were cloned in agar and overlaid with either FHS or rabbit IgG anti-sheep IgG. Colonies were examined for the presence of immunoprecipitates but none were detected. Cloning efficiencies were low and variable; from 0.5 to 8%.

Antibody secretion by hybrids was also tested by IHA. (It can be seen from Tables 3.2 and 3.3 (Chapter 3) that the IHA used was sensitive. The titres of mouse, rat, rabbit and sheep sera obtained by IHA were within 2 dilutions of those obtained by RIA). It was found that different batches of FCS had haemagglutination titres of between 4 and 64; only those exhibiting titres of less than 8 were used in the cell culture medium so that background haemagglutination was almost eliminated (some FCS haemagglutination activity disappeared during cell culture). Even so, no hybrid supernatants were found to contain anti-fluke antibody by IHA.

2.3.2 *Rat x Mouse Hybrids*

The 5 cell types listed in Section 2.3.1 could be recognised in cultures resulting from the fusion of rat spleen cells with X63 or NSI cells. Events in culture were also similar except that very few epithelial-type cells were seen. The hybrid cells were transferred to flasks about 5 weeks postfusion. From 6 fusions between 10 and 90% of wells contained hybrids.

The suitability of a haemolytic plaque screening assay of cells cloned in agar was examined by first assaying spleens from infected rats in Cunningham chambers and rat sera in haemolytic spot assays in agarose.

The haemolytic spot test yielded titres of 256 for the rabbit anti-sheep serum, 64 for the rat anti-sheep serum and between 4 and 32 for infected rat sera IR4, 5 and 6.

After suitable assay conditions were established, estimates of the number of plaque forming cells (PFC) in the spleens of rats 4 and 5 were made. In 2 experiments the numbers of PFC against FHS-coated SRBC (15 and 56 per 10^7 viable leukocytes) were not markedly above background levels using normal SRBC (9 and 49 per 10^7 viable leukocytes).

Six hybrids were cloned in agar and overlaid with FHS-coated or untreated SRBC. Although lysis was observed it was not specific for FHS and often did not coincide with a colony of cells.

Since the use of a haemolytic assay appeared to be ineffective, rat x mouse hybrid cell supernatants were screened by IHA. This test also failed to reveal any specific antibody to fluke antigens.

2.3.3 *Mouse x Mouse Hybrids*

Four mouse x mouse fusions were performed. The morphology of cells in cultured mouse x mouse fusion mixtures was

similar to that of rat x mouse fusions. Two weeks after the second fusion fibroblastic cells occupied about 70% of the wells, hybrid cells 60%, epithelial-type cells 10% and small spherical cells 20% of the wells. Few wells containing epithelial and small spherical cells lacked fibroblastic cells. A similar correlation between the occurrence of fibroblastic and hybrid cells was not evident. Wells initially lacking hybrid, fibroblastic or epithelial type cells may have eventually acquired them by cross-inoculation of wells since only one Pasteur pipette per 24 well tray was used to withdraw spent medium. Fusions 7/8 and 9/10 produced similar numbers of hybrid cells but fewer wells (35 and 60%) contained non-hybrid cell types. However, from fusion 1 only 30% of wells contained hybrids but 50% contained non-hybrid cell types (see Table 3.4, Chapter 3).

Hybrid cells from fusions 1, 2 and 7/8 were screened by IHA. Titres of hybrid and NSI supernatants and fresh culture medium ranged from 2 to 8 with one-dilution differences between some replicates.

2.4 DISCUSSION

The literature contains many modifications of the method of somatic cell hybridisation described by Galfre *et al.* (1977), all of which yield hybridomas. The modifications used in the present study were no exception. Interspecific and intraspecific hybrid cells were readily produced but the methods used for screening them failed to reveal any that secreted anti-fluke antibody.

Immunization is probably a far more effective means than infection for stimulating animals to obtain anti-parasite hybridomas. The spleen appears to be a satisfactory source of AFCs but if large animals such as sheep are to be kept alive, cells could be taken from a cannulated lymph duct. Blood has been taken from humans for fusions but this is a dilute source of AFCs.

There is strong evidence that the myeloma-like cells that survived in HAT medium after fusions of sheep spleen cells with X63 cells were interspecific hybrids. Survival in aminopterin could only have occurred if the cells were either X63 cells which reverted from HGPRT⁻ to HGPRT⁺ status or hybrid cells. The former is unlikely since such reversion is rare (Croce *et al.*, 1973; Gefter *et al.*, 1977). Moreover, in the present study all X63 cells cultured in HAT medium died. A more appropriate control would have been to have mixed X63 and spleen cells, omitted PEG treatment, then incubated the mixture in HAT. However, this was not carried out.

The finding that one culture contained sheep Ig is the strongest evidence in support of the hybrid nature of the cells. The failure of the fluorescent antibody test to reveal surface sheep Ig indicates that the cells either were secreting or did not synthesize Ig. Zagury *et al.* (1979) found that the hybrid cells they produced that were secreting Ig were surface Ig negative.

Diploid sheep cells contain 54, and X63 cells 61 to 66 (mean 63) chromosomes. The Ig-secreting sheep x mouse hybrid cells

contained fewer chromosomes than X63 cells. Nevertheless, the karyotypic instability of this cell culture suggested that it was a hybrid rather than an X63 cell because karyotypic instability is a characteristic of interspecific hybrid cells (Bernhard 1976; Ringertz and Savage 1976).

The cells presumed to be hybrids from fusions of sheep, rat and mouse spleen cells were often accompanied by other spleen-derived cell types. These cells appeared to have no effect on the survival of hybrids, gauged by the lack of correlation between the incidences of hybrid and fibroblastic cells in culture wells in each fusion. The non-hybrid cells were not characterized. However, the small, spherical cells could have been B cells responding to the FCS and mouse antigens in the cultures. These cells were not the source of sheep Ig detected in supernatants from a cell culture (Fig. 2.2) because this culture contained only hybrid cells when those supernatants were collected.

Immunoprecipitation in association with positive clones was not observed in the present study although the technique has been used successfully to detect Ig secretion by clones growing in agar (Coffino and Scharff 1971; Cook and Scharff 1977). There are several possible reasons for its inadequacy in the present instance: (1) its insensitivity, (2) FCS in the agar may have absorbed out much of the rabbit anti-sheep serum (these 2 reagents reacted in ID; Section 2.3.1), (3) the inability of many MCA to precipitate with antigens (Milstein 1980; Yelton and Scharff 1980). Furthermore, the large variation in density of clones growing in agar would make it difficult to determine which, if any, were covered by precipitates.

The limited success of the Cunningham plaque and haemolytic spot assays using cells and sera from infected rats suggested that a haemolytic plaque assay would prove to be an inadequate screening method. Nevertheless, it was considered that the haemolytic plaque assay applied to colonies of cells growing in agar might have some prospect of success because of the possible presence of higher concentrations of antibody. Moreover, this kind of technique had been used successfully by Köhler and Milstein (1975, 1976). However, this method did not detect positive clones. Another technique that used antigen-coated SRBC, IHA, also failed to detect any MCA.

The haemolytic and IHA assays may have been ineffective because the density of each antigen derived from the complex mixture of antigens in FHS on the SRBC surface was too low, even in the presence of a developing serum (rabbit anti-species Ig). This is also believed to be a reason for failures of hybridoma screening methods that rely on complement mediated cytotoxicity against lymphocytes (Milstein 1980). A means of overcoming this problem would be to add, for example, a heterospecific antiserum at subagglutinating or sublytic concentrations to the assay and rely on the low concentration of monoclonal antibody to induce haemagglutination or haemolysis. Possibly, haemolysis and IHA could have been used to screen for the secretion of non-murine Ig by hybrids by coating the SRBC targets with anti-Ig.

The failure to detect specific antibody in hybrid cell cultures may have been due to inadequate antigenic stimulation of host animals or insensitive screening methods. The latter was thought to be the more probable explanation so it was decided to proceed with a more sensitive assay - namely RIA - the results of which are presented in Chapter 3. This approach was vindicated when some supernatants shown to be negative by ID and IHA proved to be positive in RIA.

Chapter 3.

RADIOIMMUNOASSAYS FOR DETECTING HETEROGENEOUS AND
MONOCLONAL ANTIBODIES TO *FASCIOLA HEPATICA*

3.1 INTRODUCTION

There are many methods by which antibody to *F. hepatica* can be detected (see Chapter 2). However, none of these revealed hybrid cells secreting antibody with specificity for *F. hepatica*, presumably because they lacked the sensitivity required to detect the small amount of antibody secreted into the culture medium by these cells. Cultures in which hybrid cells have grown to their maximum density contain 10 to 50 μg of Ig per ml (Schneider and Eisenbarth 1979; Fazekas de St Groth and Scheidegger 1980). Another problem which emphasises the need for sensitivity is that the single antigen to which the monoclonal antibody of a hybrid cell binds is diluted by being part of a complex mixture of antigens. Therefore, when it was found that IHA was unable to detect anti-*F. hepatica* MCA it was decided to develop a more sensitive assay.

Both ELISA and RIA are becoming more frequently used in immunology because they are the most sensitive tests for antibody currently available. Moreover, they are simple to perform and amenable to automation.

The ELISAs usually performed are similar to the single indirect RIA described in Fig. 3.2 except that 1 of 2 enzymes, either alkaline phosphatase or horseradish peroxidase, is attached to the

anti-Ig instead of 125 Iodine (for descriptions and reviews see Voller, Bidwell and Bartlett 1976b; Engvall and Pesce 1978; Voller, Bartlett and Bidwell 1978; Engvall and Ruoslahti 1979; O'Beirne and Cooper 1979; Saunders 1979; Engvall 1980; Oelerich 1980; Yolken 1980). The ELISA has been used in the study of various helminth parasites (reviewed by Voller, Bartlett and Bidwell 1976a; Carlier, Bout and Capron 1979; Kagan 1979; Yolken 1980), particularly for the immunodiagnosis of *Onchocerca gutterosa* (Voller *et al.* 1976b), *O. volvulus* (Bartlett, Bidwell and Voller 1975), *Diphyllbothrium latum* (Kondo, Yoshimura, Ohnishi, Akao, Tsubota, Suzuki and Ishigooka 1980), *Echinococcus granulosus* (Bout, Dugimont, Farag and Capron 1976; Iacona, Pini and Vicari 1980; Matossian, McLaren, Draper, Bradstreet, Dighero, Kane, Mackinlay and Rickard 1979), *Trichinella spiralis* (Ruitenbergh, Steerenberg, Brosi and Buys 1974), *Taenia saginata* (Craig and Rickard 1980), *Schistosoma haematobium* (Madwar and Voller 1975), *S. japonicum* (Tanaka, Matsuda and Nosenas 1979) and *S. mansoni* (Madwar and Voller 1975; Bout *et al.* 1976; Deelder, Ruitenbergh, Kornelis and Steerenberg 1977; Hillyer and Gomez de Rios 1979; McLaren, Long, Goodgome and Lillywhite 1979; Lunde and Ottesen 1980; Rotmans and Mooij 1980), as well as for detecting *S. mansoni* circulating antigens in the sera of infected animals and humans (Madwar and Voller 1975; Ferreira *et al.* 1979; Deelder *et al.* 1980).

ELISA tests capable of detecting ovine (Lehner and Sewell 1980), bovine (Burden and Hammet 1978), rat and rabbit (Hillyer and Santiago de Weil 1979; Haroun *et al.* 1980b; Lehner and Sewell 1980) and mouse (Levine *et al.* 1980) antibodies against *F. hepatica* adult

soluble somatic antigens have been described. All these assays were used to monitor changes in serum antibody levels following infection.

Both RIA and ELISA have disadvantages in that they are time consuming (about 10 h over 3 d), expensive to perform and require the use of toxic materials to label antibody; the availability of a gamma counter usually dictates which of the 2 is chosen. These disadvantages thus delayed the start of attempts to develop an RIA for screening hybrid cells until the effectiveness of inexpensive, rapid and safe methods were assessed.

Although immunoprecipitation haemagglutination and lytic assays have been used for the detection of specific antibody secreted by hybrid cells, by far the most commonly employed methods have been binding assays modified to provide for maximum sensitivity (see Section 1.3.3). Radioisotopes are often incorporated in such assays because they contribute greatly to sensitivity (Galfre *et al.* 1977; Koprowski *et al.* 1977). The solid phase RIA using soft PVC 96-well trays is a popular method for screening hybrid cells because soluble antigens readily bind to PVC. Soft PVC trays produce clearer results and higher titres than polystyrene trays (Hillyer and Santiago de Weil 1979). The antigen-coated wells enable many samples to be rapidly screened for the presence of antibody. This screening method has been used for the detection of human monoclonal Ig having no defined specificity (Levy and Dilley 1978) and MCA to haptens (Kennett *et al.* 1978; Olsson and Kaplan 1980), synthetic polypeptides (Pierres *et al.*

1979), mouse IgG (Oi *et al.* 1978; Parks *et al.* 1979) and IgM (Kennett *et al.* 1978), glucose-6-phosphate dehydrogenase (Damiani *et al.* 1980), guanylate cyclase (Lewicki *et al.* 1980), rat retina (Barnstable 1980), influenza (Koprowski *et al.* 1977; Effros *et al.* 1979), *P. yoelii* (Kim *et al.* 1980), *T. gondii* (Sethi *et al.* 1980), *E. granulosus*, *T. hydatigena* and *T. ovis* (Craig *et al.* 1980) and *S. japonicum* (Mitchell *et al.* 1981).

Levy and Dilley (1978) used a direct RIA; the iodinated tracer binding to the solid phase (PVC) via only one other type of molecule (Fig. 3.1). The other solid phase RIAs listed above were indirect RIAs. The iodinated tracer in an indirect RIA binds to the solid phase via 2 other types of molecule; the antigen and the antibody which binds to it (Fig. 3.2).

Originally, exclusive use was made of iodinated anti-Ig in indirect RIAs (Tsu and Herzenberg 1980) but, more recently, many workers have replaced iodinated anti-Ig with iodinated protein A (Fig. 3.3). Protein A is extracted from the cell wall of the bacterium, *Staphylococcus aureus* Cowan I strain. Protein A binds to many Igs, including human IgG₁, IgG₂, IgG₄, IgA₂ and some IgM, mouse IgG_{2a}, IgG_{2b} and some IgG₁, guinea pig IgG₁ and IgG₂, rabbit IgG and echidna IgG (Goding 1978), purified human IgE (Inganas, Johansson and Bennich 1980), sheep IgG₂ and goat IgG (Goding 1978; Goudswaard, Van der Donk, Noordizij, Van Dam and Vaerman 1978; Delacroix and Vaerman 1979; Duhamel, Meezan and Brendel 1980), some sheep IgG₁ (Goudswaard *et al.* 1978; Howell, pers. comm.), bovine IgG₂ and dog and cat IgG and some

Fig. 3.1

The direct, competitive RIA

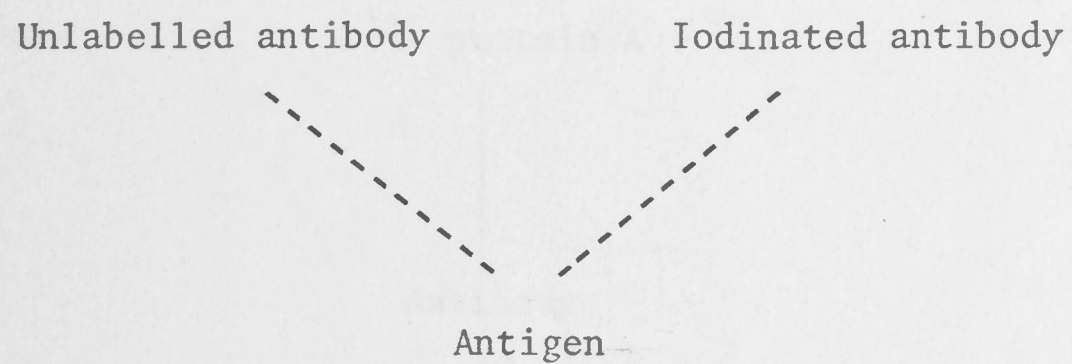


Fig. 3.2

The single indirect RIA

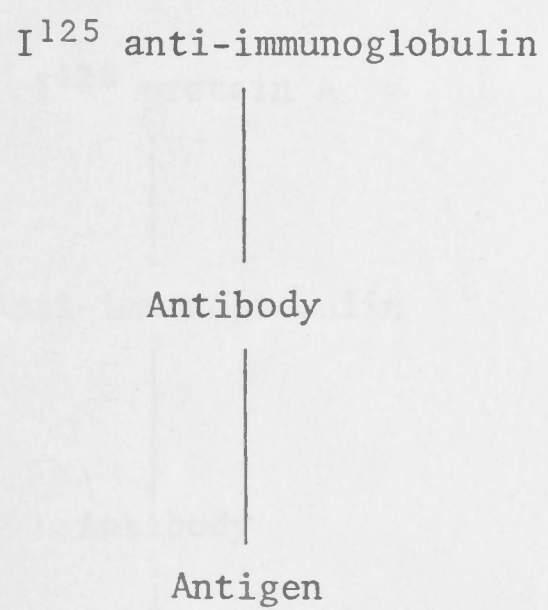


Fig. 3.3

The protein A single indirect RIA

 I^{125} protein A

Antibody

Antigen

Fig. 3.4

The double indirect RIA

 I^{125} protein A

Anti-immunoglobulin

Antibody

Antigen

IgA and IgM (Goudswaard *et al.* 1978), rat IgG₁ and IgG_{2c} (Medgyesi, Füst, Gergely and Bazin 1978; Rousseaux, Picque, Bazin and Biserte 1981) and some rat IgM and IgA (Medgyesi *et al.* 1978). Protein A has been used most effectively in RIAs of human and rabbit sera because the dominant isotypes of these species are strongly bound by it.

Iodinated protein A single indirect RIAs (Fig. 3.3) have been used to assay antibodies to cell surface antigens (eg. Dorval, Welsh and Wigzell 1975; Welsh, Dorval and Wigzell 1975; Langone, Boyle and Borsos 1977) and against soluble antigens when they were insolubilised on PVC wells (Marier, Jansen and Andriole 1979), polystyrene tubes (Avraham, Spira, Gorsky and Sulitzeanu 1980), agarose beads (Langone *et al.* 1977; Lambden and Watt 1978; Langone 1978, 1980), paper discs (Cleveland, Richman, Oxman, Wickham, Binder and Worthen 1979) and RBC (Romagnani, Del Prete, Guidizi, Almerigogna and Ricci 1979).

Iodinated protein A single indirect RIAs have been incorporated into hybridoma technology. Several workers have used such assays to detect monoclonal mouse antibodies to cell surface antigens (Hämmerling *et al.* 1978; Kennett *et al.* 1978; Schneider and Eisenbarth 1978) and soluble antigens (Olsson and Kaplan 1980; Businaro *et al.* 1981). However, this type of assay has the disadvantage that it cannot detect antibody isotypes which are not bound by protein A, such as rat IgG_{2a}. Moreover, sensitivity will be diminished if the antibody the assay is required to detect is of a subclass poorly bound by protein A, such as mouse or sheep IgG₁. The

problem is exacerbated by the fact that the isotypes referred to in these examples are the major ones produced during humoral immune responses in rats, mice and sheep.

Brown *et al.* (1979) have successfully overcome this problem by inserting a rabbit IgG anti-mouse IgG (RAM) in the assay to produce a double indirect RIA (Fig. 3.4). Since protein A has a high affinity for rabbit IgG, these workers found that the sensitivity of their assay for detecting monoclonal mouse antibodies to cell surface antigens was greatly increased. In theory, adaptation of this assay for the detection of antibodies from any other species of animal, such as the rat or sheep, would simply involve substitution of RAM by rabbit antibodies to the appropriate Igs of the chosen animal. As yet, however, only mouse antibodies have been assayed with a double indirect RIA. Moreover, there are no reports of double indirect RIAs using soft PVC as the solid phase.

In helminthology, RIAs have been used for the detection of antibodies to schistosomes (Schinski, Clutter and Murrell 1976; Pelley, Warren and Jordan 1977), *T. spiralis* (Belozyorov, Bessonov, Vishnyauskas and Shekhovtsov 1978; Biancifiori, Frescura, Gialletti and Morozzi 1980), *Nematospiroides dubius* (Day, Howard, Prowse, Chapman and Mitchell 1979), *Ascaris lumbricoides* (O'Donnell and Mitchell 1980), *M. corti* (Mitchell *et al.* 1979) and other cestodes (Craig *et al.* 1980) and of *S. mansoni* antigen in serum (Carlier *et al.* 1980). These assays, except the direct RIA developed by Belozyorov *et al.* (1978), were single indirect RIAs. None of them employed soft

PVC as the solid phase except Biancifiori *et al.* (1980) and Craig *et al.* (1980) and none used iodinated protein A as the tracer.

In order to screen hybrid cells for those secreting anti-*F. hepatica* antibody solid phase RIAs were developed. A single indirect assay incorporating iodinated anti-Ig (Fig. 3.5) was used until replaced by a double indirect RIA (Fig. 3.6). The latter could also be used for the detection of anti-*F. hepatica* antibodies in the sera of mice and, with appropriate reagents, rats or sheep. Iodinated protein A was also employed in a single indirect RIA (Fig. 3.3) to test a rabbit antiserum to fluke antigens.

3.2 MATERIALS AND METHODS

3.2.1 Antigens

Methods of producing adult fluke somatic antigen, FHH, the soluble fraction of FHH, FHS, and incubation products of adult flukes (FIP) and excysted metacercariae (MFIP) are described in Section 2.2.1.

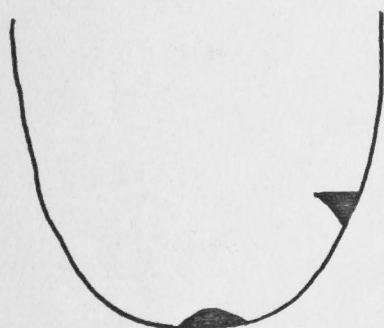
3.2.2 Antibodies

The sources of antibodies to *F. hepatica* antigens used to develop the RIAs are described in Section 2.2.2 and summarised in Tables 3.2 and 3.3. Most of these sera were produced coincidentally to the antigenic stimulation of animals before fusion of their spleen cells with mouse myeloma cells.

Fig. 3.5. Diagram of events in a solid phase single indirect RIA using I^{125} RAM and soft PVC trays to detect mouse antibodies to *F. hepatica* antigens.

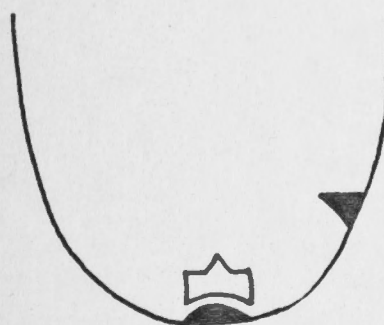
THE SINGLE INDIRECT RADIOIMMUNOASSAY

1



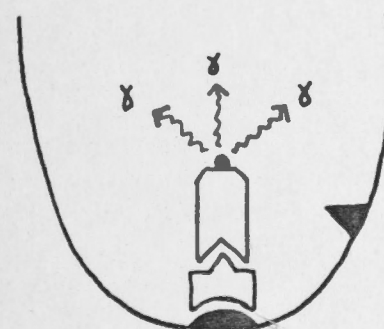
F.hepatica antigens

2



Mouse anti-*F.hepatica* antibody

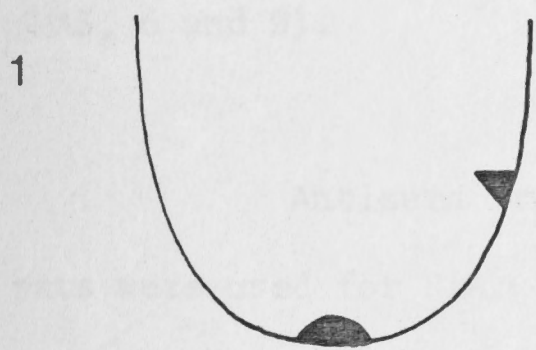
3



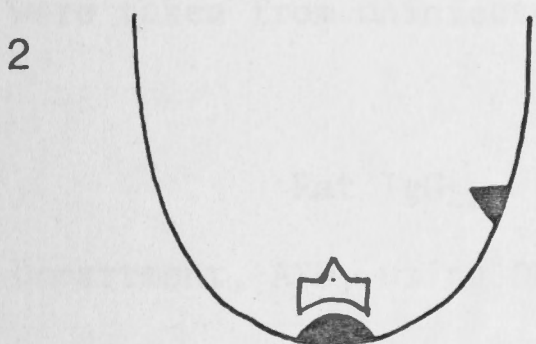
¹²⁵Iodine-labelled rabbit IgG anti-mouse IgG

Fig. 3.6. Diagram of the I^{125} protein A solid phase double indirect RIA used to detect antibodies to *F. hepatica* antigens.

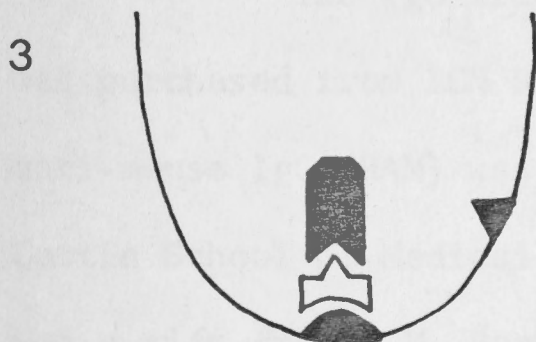
THE DOUBLE INDIRECT RADIOIMMUNOASSAY



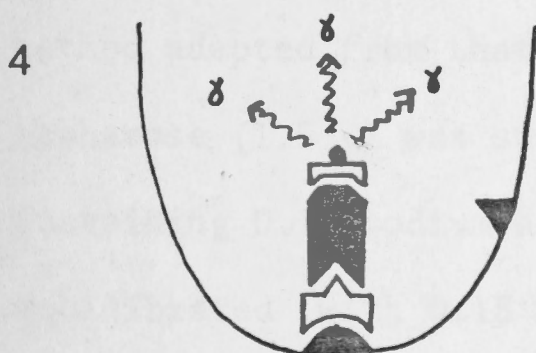
F. hepatica antigens



Mouse anti-*F. hepatica* antibody



Rabbit IgG anti-mouse IgG



Iodinated *Staphylococcus*

aureus protein A

Seven mouse antisera were produced; 4 by injections of FHH (MA1, 2, 3 and 4) and 3 by injections of FHS-coated RBC (MA5, 6 and 9).

Antisera from 2 immunized rats and sera from 3 infected rats were used for RIA. These were collected following the injection of immune precipitated adult (RA1) or metacercarial (RA2) antigens or infection (IR4, 5 and 6) as described in Section 2.2.2. Normal sera were taken from uninfected, non-immunized animals.

Rat IgG_{2a} was purified, by A.M. Barton of the Zoology Department, ANU, using DEAE Sephadex A-50, from the sera of rats which carried an IgG_{2a}-secreting myeloma.

The IgG fraction of a rabbit antiserum to sheep IgG (RAS) was purchased from ICN Radiochemicals (California). The rabbit IgG anti-mouse IgG (RAM) was a gift from Professor G.L. Ada of the John Curtin School of Medical Research, ANU. A rabbit antiserum to rat IgG was a gift from A.M. Barton of the Zoology Department, ANU. The IgG fraction of this rabbit serum, RAR, was obtained by affinity chromatography on a protein A sepharose (Pharmacia) column using a method adapted from that of Ey, Prowse and Jenkin (1978). Protein A sepharose (1.5 g) was swollen overnight at 4°C in 0.1 M PBS, pH 8.0, containing 0.1% sodium azide. The 5 ml column (13 mm x 70 mm) was equilibrated with 0.15 M phosphate buffer, pH 8.0, containing 0.05% NaN₃. One ml of rabbit antiserum was added in 2 ml of the same buffer, which was also used to wash unbound material through the column. The rabbit IgG was eluted with 0.1 M sodium citrate buffer, pH 3.0, then

concentrated by dialysis against 20% PEG (Grade II, Calbiochem) in PBS. Goding (1976b), Goudswaard *et al.* (1978) and Miller and Stone (1978) have shown that rabbit IgG isolated from serum in one step by protein A sepharose affinity chromatography is both antigen binding and antigenic.

3.2.3 Iodination

Iodination of *S. aureus* protein A (Sigma) and RAM by the chloramine T method (Hunter and Greenwood 1962) was performed by a variation of the method of Bolton (1977). To 0.5 mCi NaI¹²⁵ (100 mCi/ml, specific activities 12 to 17 mCi/ μ g; Radiochemical Centre, Amersham) were added 10 μ l 0.25 M phosphate buffer (pH 7.5), 50 μ g protein (either protein A or RAM) in 50 μ l 0.05 M phosphate buffer and 50 μ l 0.5% chloramine T in 0.05 M phosphate buffer. After mixing, 0.1 ml 0.12% sodium metabisulphite in 0.05 M phosphate buffer then 0.6 ml 0.2% KI in 2% FCS in 0.05 M phosphate buffer were added. Initially, free iodine was removed by overnight dialysis against 2 changes of 200 ml PBS. Later, free iodine was separated from iodinated protein A more rapidly by gel filtration (Bolton 1977). This entailed applying the reaction mixture to a 20 ml (25 mm x 50 mm) column of 5 g Sephadex G25 pretreated with 1 mg BSA in 1 ml 0.05 M phosphate buffer. The first 7 ml of eluate was discarded then the next 4 ml retained as iodinated protein A in 2% FCS in PBS. Specific activities of the 2 iodinated proteins were between 2 and 5 μ Ci/ μ g.

3.2.4 *Immunoassay*

This method is a modification of that described by Klinman, Pickard, Sigal, Gearhart, Metcalf and Pierce (1976). Details of experiments used to determine optimal reagent concentrations and incubation times appear in Section 3.3. The method described here and summarised in Fig. 3.7 incorporates these optima. All incubations were at room temperature except where stated.

Wells of flexible 96 well PVC microtitre trays (Cook, Dynatech) were coated with fluke antigens by treatment for 2 h with 100 μ l FHS, FIP or MFIP at 15 μ g/ml PBS. After rinsing with 1% FCS in 0.9% NaCl (NSF) wells were filled with 10% FCS in PBS and left a further 30' to ensure that any unbound sites on the PVC were occupied by non-Ig molecules. The trays were rinsed with NSF then placed in a dessicator at 4°C until use, or disposal after 5 d. Wells were then incubated with 50 μ l serum or cell culture supernatant for 2 h and rinsed with NSF.

If a double indirect RIA was required the wells were incubated with 50 μ l secondary antibody (RAM, RAR or RAS) at 5 μ g/ml PBS for 2 h and rinsed with NSF (Step 5, Fig. 3.7). Alternatively, if a single indirect assay was required this step was omitted.

The reagent used in the final incubation depended on the type of assay. Iodinated RAM at 1 μ g/ml 1% FCS, 0.1% Tween 20 in PBS (50 μ l, about 10^5 cpm) was added if a single indirect assay was carried out (Step 6a, Fig. 3.7). Otherwise, each well received 50 μ l

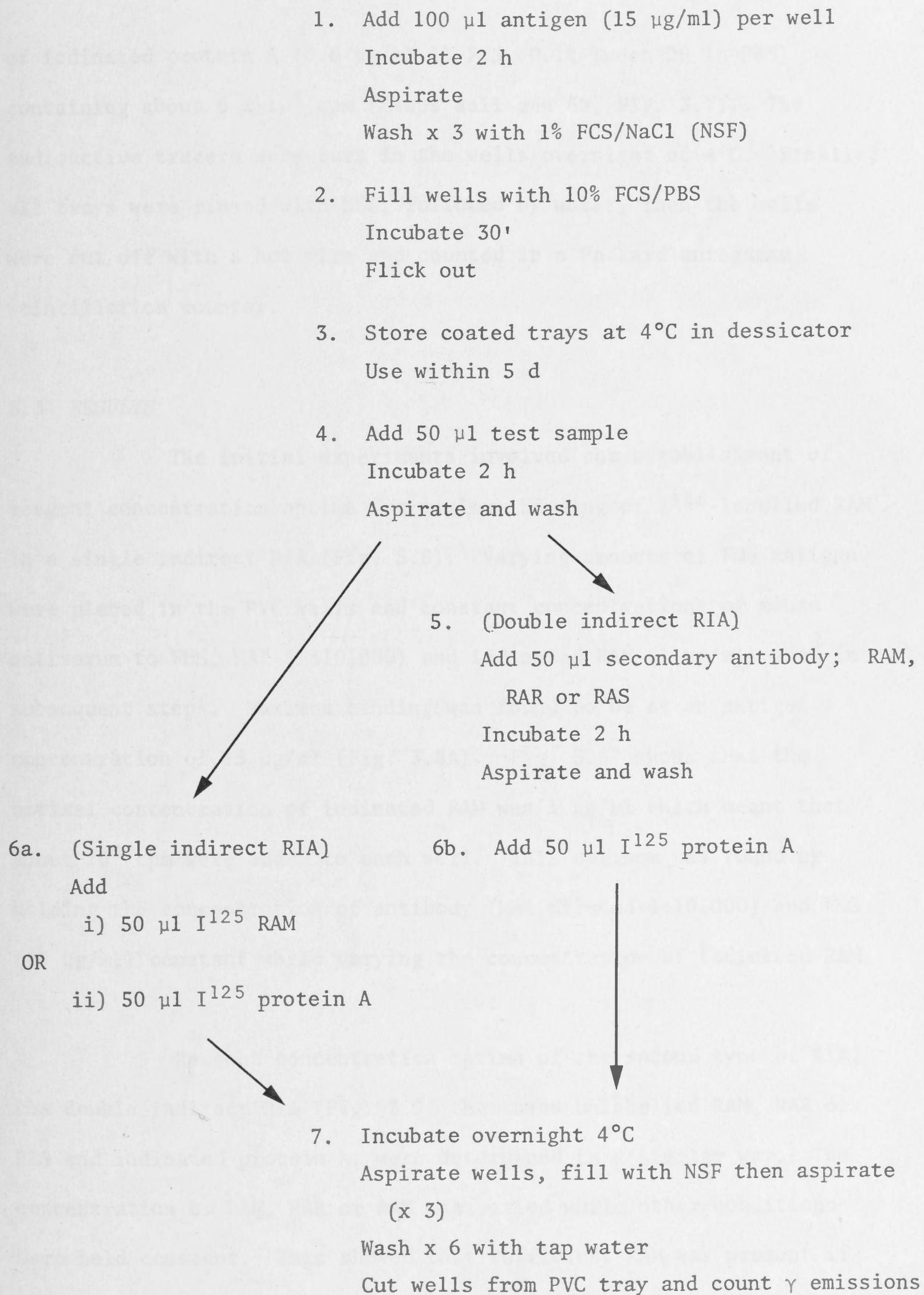


Fig. 3.7. Flow chart of the procedure employed to detect antibodies to *F. hepatica* antigens by solid phase RIA.

of iodinated protein A (0.6 $\mu\text{g/ml}$ 1% FCS, 0.1% Tween 20 in PBS) containing about 6×10^4 cpm (Steps 6a_{ii} and 6b, Fig. 3.7). The radioactive tracers were left in the wells overnight at 4°C. Finally, all trays were rinsed with NSF, followed by water, then the wells were cut off with a hot wire and counted in a Packard autogamma scintillation counter.

3.3 RESULTS

The initial experiments involved the establishment of reagent concentration optima for maximum binding of I^{125} -labelled RAM in a single indirect RIA (Fig. 3.8). Varying amounts of FHS antigen were placed in the PVC wells and constant concentrations of mouse antiserum to FHH, MA1 (1:10,000) and iodinated RAM (1 $\mu\text{g/ml}$) used in subsequent steps. Maximum binding was found to be at an antigen concentration of 15 $\mu\text{g/ml}$ (Fig. 3.8A). Fig. 3.8B shows that the optimal concentration of iodinated RAM was 1 $\mu\text{g/ml}$ which meant that about 10^5 cpm were added to each well. This optimum was found by holding the concentration of antibody (MA1 diluted 1:10,000) and FHS (15 $\mu\text{g/ml}$) constant while varying the concentration of iodinated RAM.

Reagent concentration optima of the second type of RIA, the double indirect RIA (Fig. 3.9) that uses unlabelled RAM, RAR or RAS and iodinated protein A, were determined in a similar way. The concentration of RAM, RAR or RAS was varied while other conditions were held constant. This showed that sufficient RAM was present if added at about 5 $\mu\text{g/ml}$ (Fig. 3.9A). Following similar procedures, 10 $\mu\text{g/ml}$ was found to be a suitable concentration of both RAR and RAS.

Like iodinated RAM, iodinated protein A was used at a sub-saturating concentration, 600 ~~ng~~ ^{ng}/ml (6×10^4 cpm per well; Fig. 3.9B).

It was found that omission of the incubation with 10% FCS/PBS, designed to occupy unbound sites on the PVC before the addition of antibody, had no effect on the number of cpm bound.

Time course experiments showed that: i) most of the antigen is rapidly adsorbed to the PVC wells (Fig. 3.10A) and the process is completed after 30', ii) antibody binds maximally after 1 h (Fig. 3.10A) and iii) RAM requires about 90' to give maximum binding, as does iodinated protein A (Fig. 3.10B).

Table 3.1 lists the low levels of radioactivity bound to wells after various control treatments. The background radiation was about 20 cpm. Little radioactivity was bound when serum or iodinated tracer was omitted. About 400 cpm were bound by most normal mouse serum controls; the exception was that normal serum bound 700 cpm when the wells were later incubated with RAM then iodinated protein A. When the antigen incubation was omitted the mouse antiserum MA1 bound 120, 300 or 500 cpm when subsequent incubations were iodinated protein A, iodinated RAM or RAM followed by iodinated protein A respectively. Antigen-omitted, normal rat serum and normal rat IgG_{2a} controls, using RAR on rat sera, exhibited a high level of binding (about 2,400 cpm) compared with similar double indirect RIAs using RAM (up to 550 cpm; Table 3.1B, C). It was found that RAS did not crossreact with RAM or RAR and that in a single indirect RIA, when RAS was omitted,

iodinated protein A did not bind the infected sheep serum (Table 3.1Di). There were 2 other sheep serum controls. Only 400 cpm was bound by the infected sheep serum when antigen was omitted in a double indirect RIA and normal sheep serum bound 500 cpm in the same type of assay when the antigen incubation was included (Table 3.1D).

A sample titration of mouse antiserum MA4 is presented in Fig. 3.11. The titre is taken as the reciprocal of the serum dilution which binds a level of radioactivity twice that bound by normal serum at the same dilution. The titre of MA4 was 90,000.

The titres of various sera in single and double indirect RIAs are listed in Tables 3.2 and 3.3. In some cases titres measured by IHA (see Section 2.2.7) are included for comparison.

Similar amounts of antibody seem to be present in sera from mice injected with FHH or FHS-coated RBC (Table 3.2). About 3 to 4% of the iodinated RAM added was bound at a mouse antiserum dilution of 1:100. The use of RAM plus iodinated protein A greatly increased the degree of binding by MA1, diluted 1:100, to nearly 50% of the cpm added. There was also an increase in radioactivity bound by normal mouse serum, from 400 cpm in the single to 700 cpm in the double indirect RIA (Table 3.1Bii). The mouse antisera also bound *F. hepatica* incubation products (FIP and MFIP) in RIAs.

Pooled serum taken from mice 18 d after infection with 5 metacercariae yielded a titre of 150 (Table 3.2). Sera from infected rats also bound little iodinated tracer (about 2,000 cpm when diluted 1:100) in single indirect RIAs which employed iodinated RAM or iodinated protein A or in a double indirect RIA with a heterologous second antibody, RAM (Table 3.3A). Higher levels of binding were produced in a homologous (RAR) double indirect RIA but binding by normal serum and normal IgG_{2a} was high (Table 3.1C).

Assay of the antiserum to immune complexed adult antigens, RA1, showed that it bound FHS (Table 3.3A). The other rat antiserum (RA2), to immune complexed metacercarial antigens, bound to FHS with a very low titre of 32. The use of RAR as the second antibody had little effect on sensitivity but the use of metacercarial incubation products (MFIP) as antigen produced higher levels of specific binding by RA2 (Table 3.3A).

The rabbit antiserum proved to be potent, exhibiting a titre of 150,000. Almost 50% of the iodinated protein A tracer was bound directly by this antiserum at a dilution of 1:100.

It was found that RAS followed by iodinated protein A could be used to titre infected sheep serum (Table 3.3C).

Having established conditions which produced a sensitive RIA, supernatants from mouse x mouse hybrid cells were tested. Initially, iodinated RAM was used but was replaced by the RAM plus

iodinated protein A method. Fig. 3.12 shows a part of one such test, performed by the former method 3 weeks after fusion of CBA spleen cells with NSI cells (mouse x mouse fusion 7/8; Section 2.2.3).

Iodinated RAM, culture medium, FCS and X63 cell supernatants bound about 100 cpm. Supernatants from most post-fusion cell cultures bound about 100 cpm but some bound more than 200 cpm. The latter supernatants, since they bound greater than double the control level of radioactivity, were assumed to contain antibody. These positive supernatants were then tested for their ability to bind PVC in the absence of antigen (Fig. 3.12). If this ability was lacking it was concluded that the sample contained anti-*F. hepatica* antibody. Of the 24 cell culture supernatants shown in Fig. 3.12, 2 bound FHS and 1 bound PVC directly.

All mouse x mouse hybrid cell cultures were tested by RIA. The proportions of these that were positive were 15, 20, 15 and 25% from the 4 fusions (Table 3.4). Stored supernatants from sheep and rat x mouse hybrids were all negative.

Fig. 3.8. Single indirect RIA. Titrations to determine optimum concentrations of (A) *F. hepatica* antigen FHS and (B) I^{125} RAM.

RADIOIMMUNOASSAY TITRATIONS

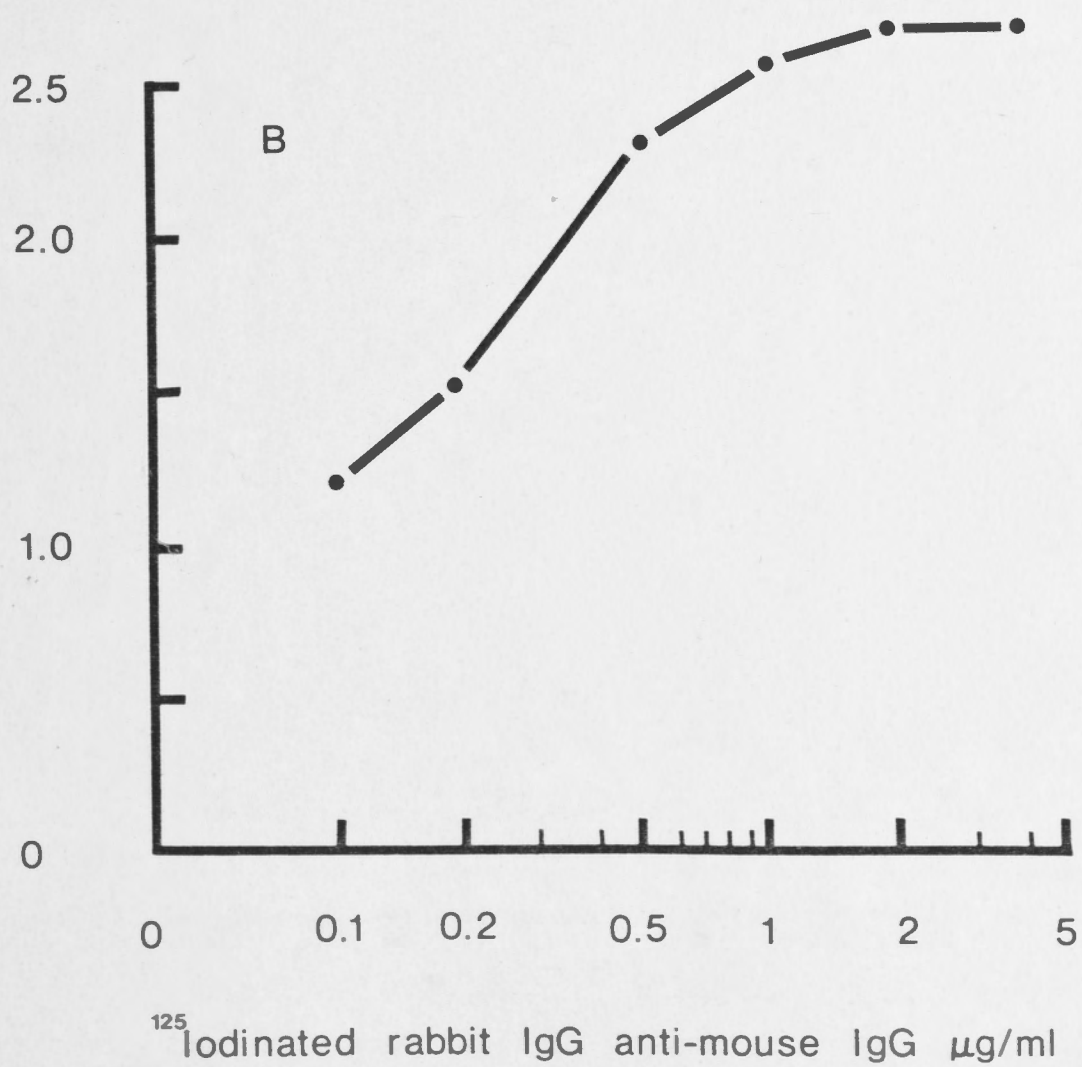
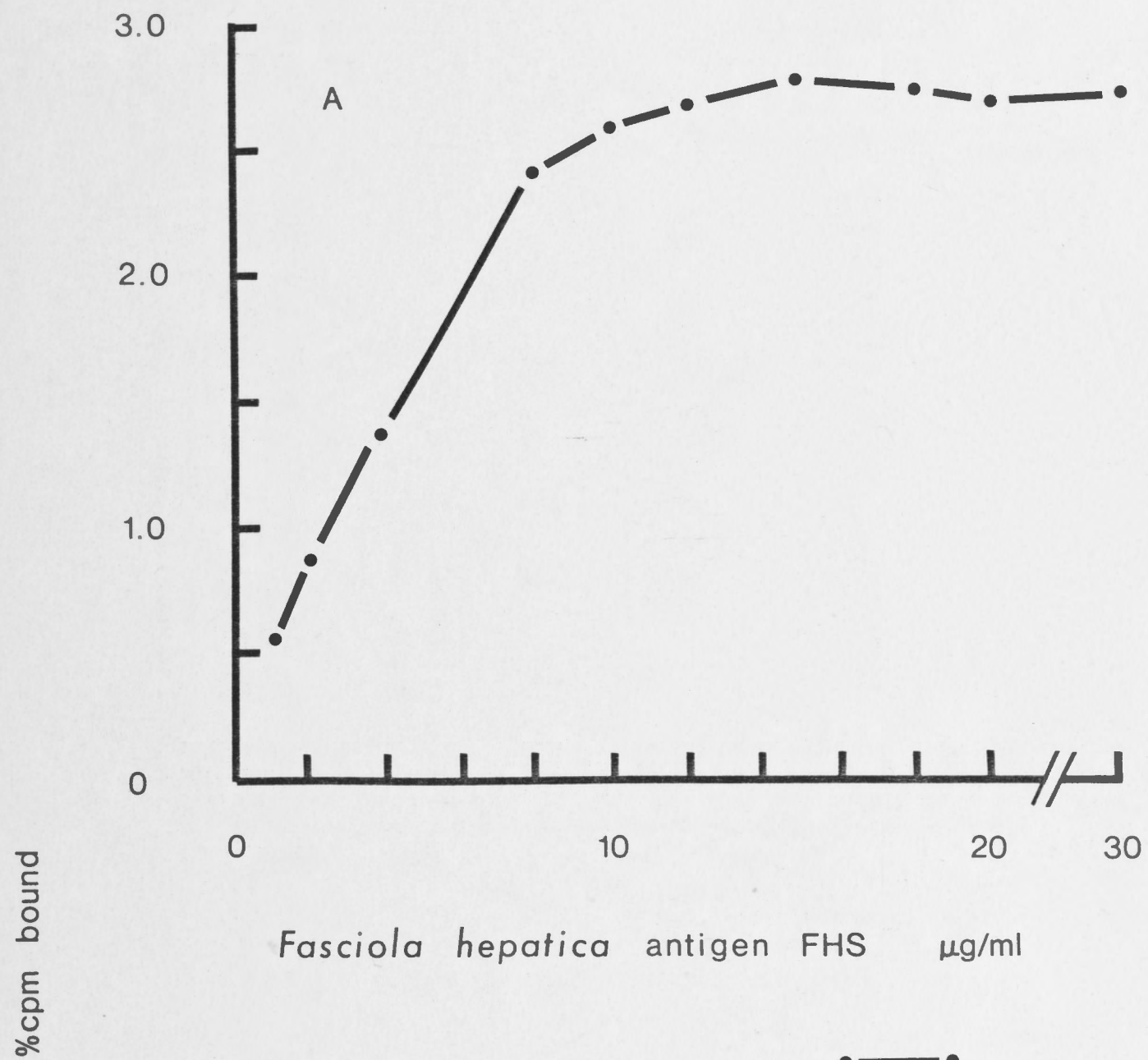


Fig. 3.9. Double indirect RIA. Titrations to determine optimum concentrations of (A) RAM and (B) I^{125} protein A.

RADIOIMMUNOASSAY TITRATIONS

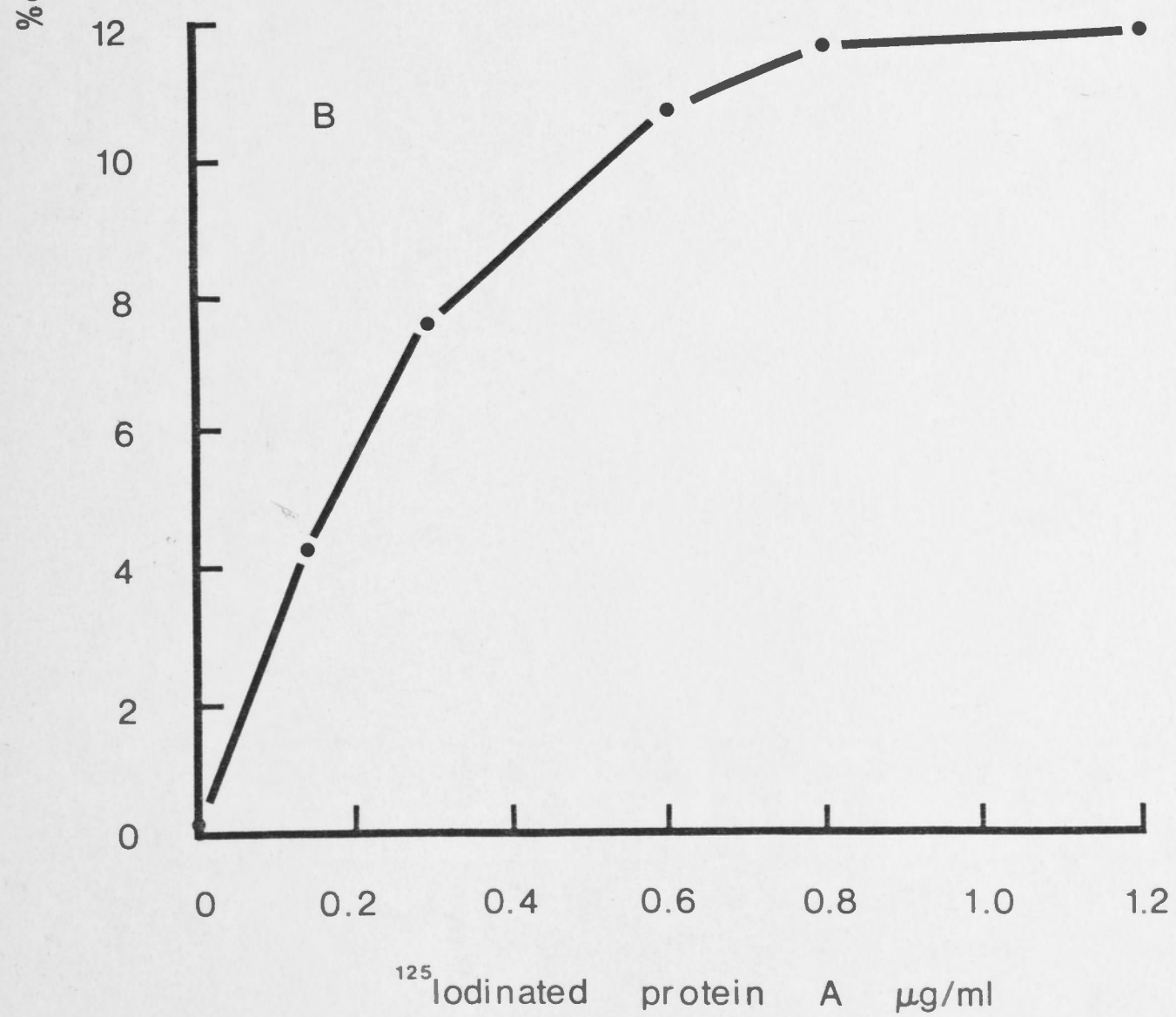
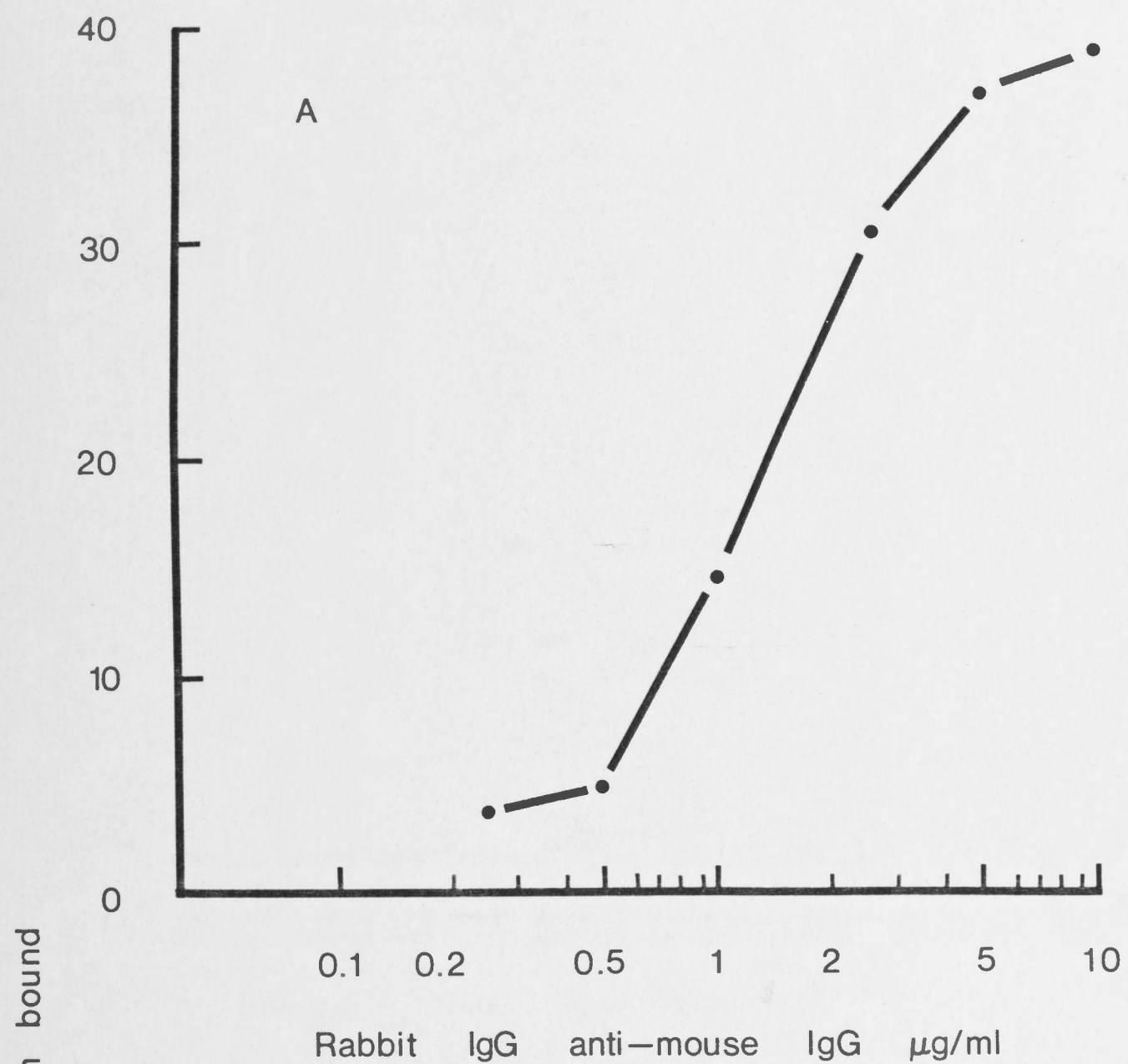


Fig. 3.10. Determinations of optimum times of incubation of PVC trays with (A) *F. hepatica* antigen FHS and mouse antiserum MA1 and (B) secondary antibody RAM and I¹²⁵ protein A.

RADIOIMMUNOASSAY TIME COURSE EXPERIMENTS

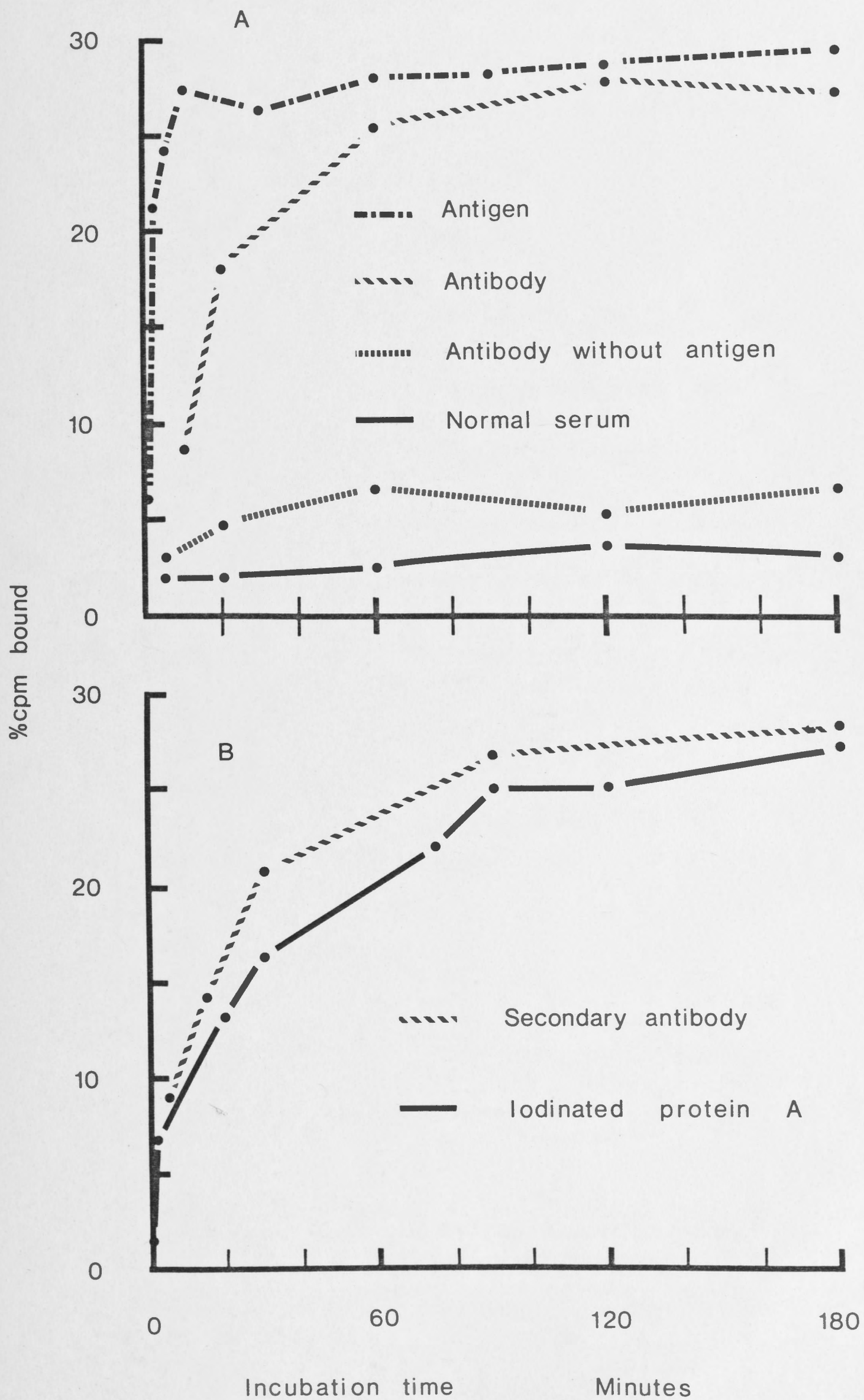


Fig. 3.11. Determination of the titre of a mouse antiserum to the fluke antigen preparation FHS (MA4) by I^{125} protein A solid phase double indirect RIA.

TITRE DETERMINATION BY RADIOIMMUNOASSAY

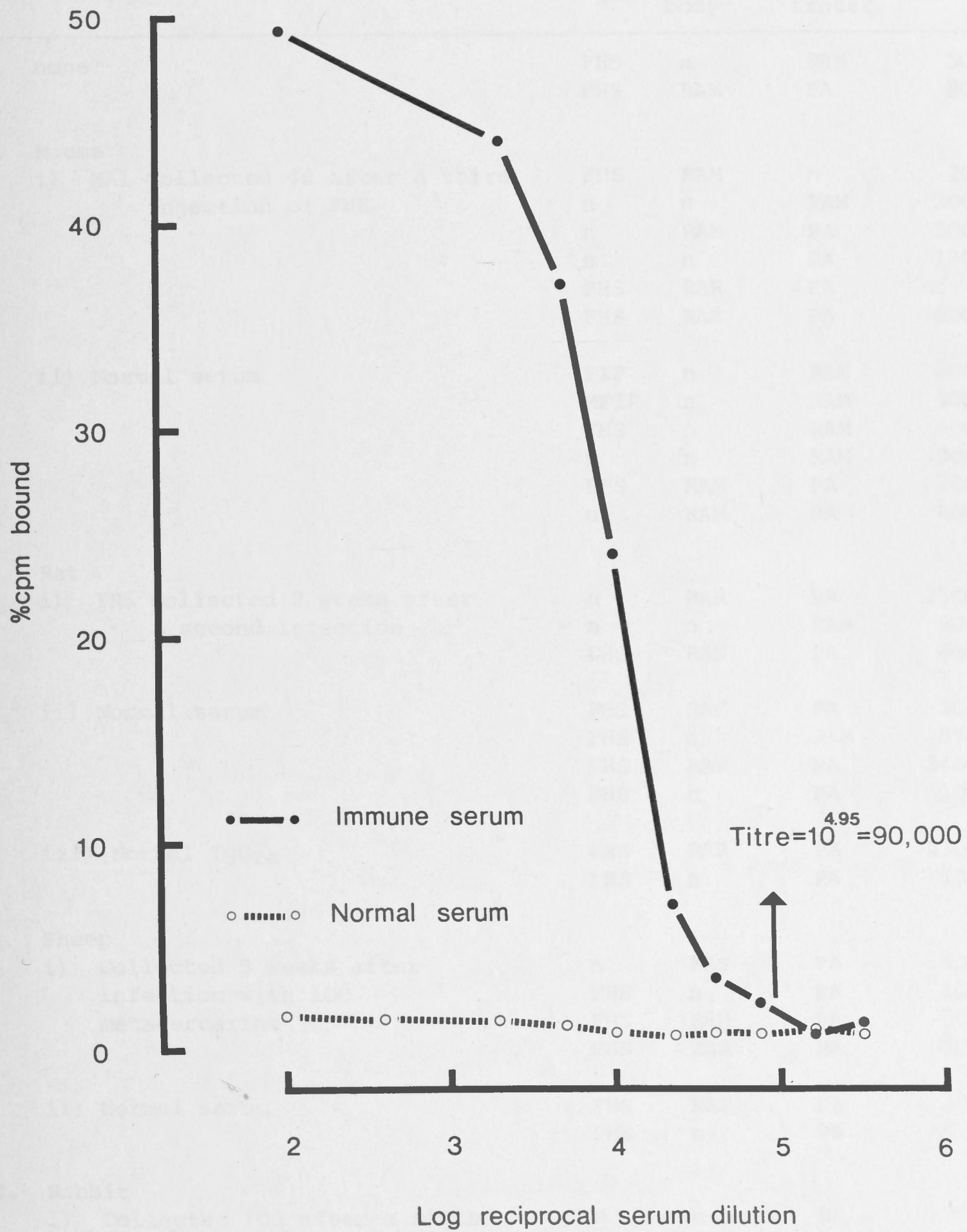


Table 3.1

CONTROL INCUBATIONS USED IN RADIOIMMUNASSAYS

Serum, diluted 1:100	Anti-gen	Second anti-body	Iodin-ated tracer	cpm bound
A. none	FHS	n	RAM	50
	FHS	RAM	PA	80
B. Mouse				
i) MA1 Collected 4d after a third injection of FHH	FHS	RAM	n	20
	n	n	RAM	300
	n	RAM	PA	500
	n	n	PA	120
	FHS	RAR	PA	nd
	FHS	RAS	PA	600
ii) Normal serum	FIP	n	RAM	400
	MFIP	n	RAM	400
	FHS	n	RAM	400
	n	n	RAM	200
	FHS	RAM	PA	700
	n	RAM	PA	400
C. Rat				
i) IR5 Collected 2 weeks after a second infection	n	RAR	PA	2500
	n	n	RAM	500
	FHS	RAS	PA	400
ii) Normal serum	FHS	RAM	PA	200
	FHS	n	RAM	550
	FHS	RAR	PA	2400
	FHS	n	PA	100
iii) Normal IgG _{2a}	FHS	RAR	PA	2300
	FHS	n	PA	100
D. Sheep				
i) Collected 9 weeks after infection with 100 metacercariae	n	RAS	PA	400
	FHS	n	PA	100
	FHS	RAM	PA	500
	FHS	RAR	PA	500
ii) Normal serum	FHS	RAS	PA	500
	FHS	n	PA	nd
E. Rabbit				
i) Collected 10d after a sixth injection of FHH	n	n	PA	300
	FHS	n	RAM	250
ii) Normal serum	FHS	n	PA	400
	FHS	RAS	PA	nd

n = none

nd = not done

PA = protein A

Table 3.2

TITRES OF MOUSE SERA TESTED BY RADIOIMMUNOASSAY AND INDIRECT HAEMAGGLUTINATION

Serum	Antigen	Second anti-body	Iodin-ated tracer	cpm bound at 1:100 ($\times 10^{-3}$)	RIA titre ($\times 10^{-3}$)	IHA titre ($\times 10^{-3}$)
MA1 4d after a third injection of FHH	FHS	n	RAM	4	80	32
	FHS	n	PA	1.3	nd	
	FHS	RAM	PA	28	120	
	FIP	n	RAM	3.8	80	
	MFIP	n	RAM	nd	30	
	MFIP	RAM	PA	2.5	20	
MA2 4d after a second injection of FHH	FHS	n	RAM	3	30	nd
MA3 10d after a third injection of FHH	FHS	n	RAM	5	100	15
MA4 As for MA3	FHS	n	RAM	4.3	90	nd
MA5 8d after a third injection of FHS - coated RBC	FHS	n	RAM	2.8	30	nd
MA6 As for MA5	FHS	n	RAM	3.5	60	nd
MA9 3d after a third injection of FHS - coated RBC	FHS	n	RAM	3.4	40	16
Taken 18d after infection with 5 metacercariae	FHS	n	RAM	0.8	0.15	0.256

n = none; nd = not done; PA = protein A

Table 3.3

TITRES OF SERA TESTED BY RADIOIMMUNOASSAY AND INDIRECT HAEMAGGLUTINATION

Serum	Antigen	Second anti-body	Iodin-ated tracer	cpm bound at 1:100 ($\times 10^{-3}$)	RIA titre ($\times 10^{-3}$)	IHA titre ($\times 10^{-3}$)
A. RAT						
RA1 7d after a second injection of immune precipitated adult antigens.	FHS	n	RAM	1.5	0.8	nd
	FHS	RAR	PA	3.0	0.08	
	MFIP	RAR	PA	8	0.4	
RA2 7d after a second injection of immune precipitated metacercarial antigens	FHS	n	RAM	0.4	0.032	nd
	FHS	RAR	PA	2.7	0.04	
	MFIP	RAR	PA	9	0.5	
IR4 20d after a third infection	FHS	n	RAM	2	nd	0.032
	FHS	n	PA	2	nd	
IR6 32d after a third infection	FHS	RAR	PA	16	nd	0.256
IR5 15d after a second infection	FHS	n	RAM	1.7	nd	0.128
	FHS	RAM	PA	2	nd	
	FHS	n	PA	0.6	nd	
	FHS	RAR	PA	7	0.3	
B. RABBIT						
Taken 10d after a sixth injection of FHH	FHS	n	PA	29	150	80
C. SHEEP						
Taken 9 weeks after an infection of 100 metacercariae	FHS	RAS	PA	3	2	2

n = none; nd = not done; PA = protein A

Fig. 3.12. An example of applying the I^{125} RAM single indirect RIA to screening hybrid cell supernatants. The supernatants were from mouse x mouse fusion 7/8.

RADIOIMMUNOASSAY OF HYBRID CELLS

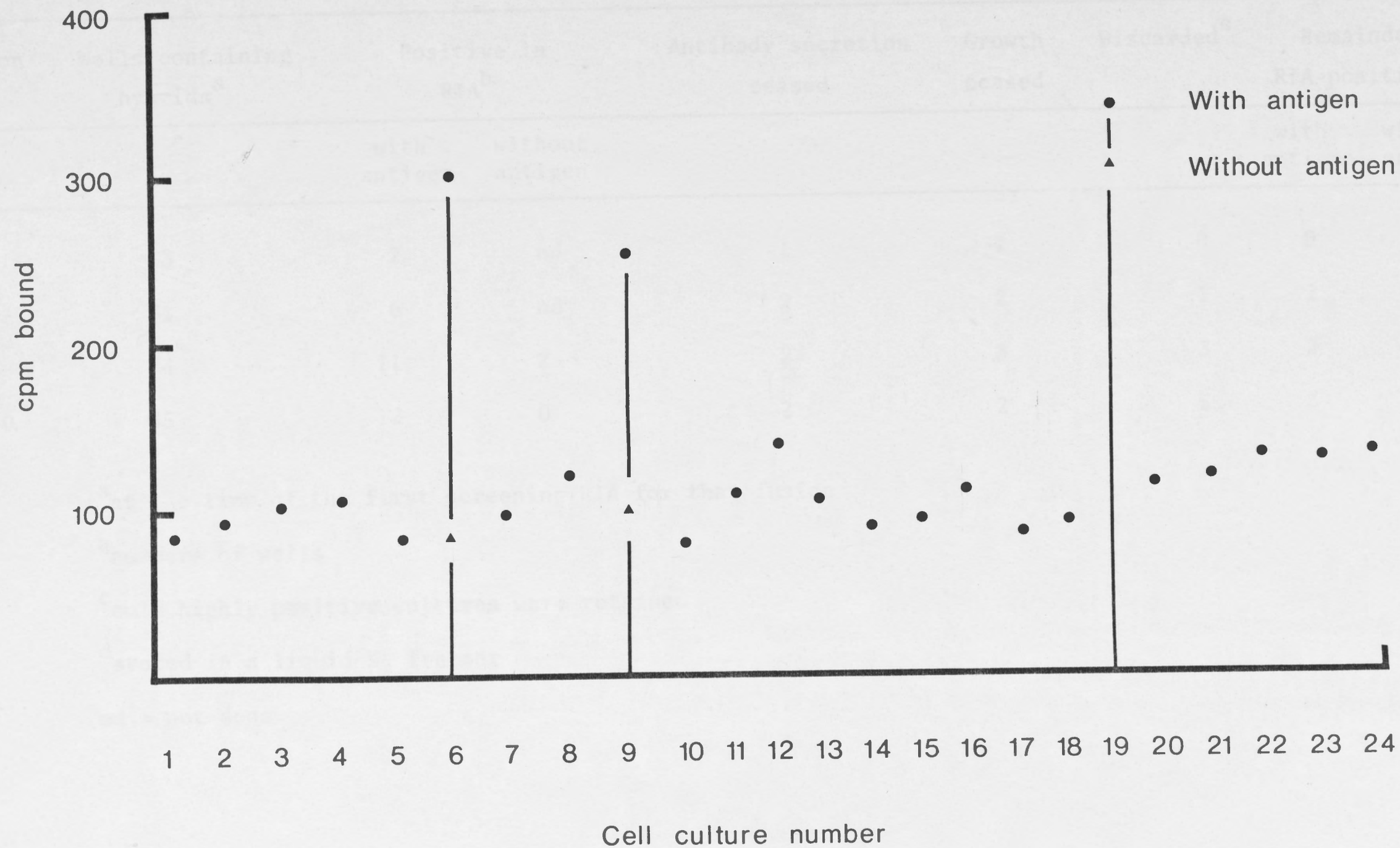


Table 3.4 YIELDS OF HYBRID CELLS FROM MOUSE X MOUSE CELL FUSIONS, NUMBERS SECRETING ANTIBODY AND THEIR FATE.

Fusion	Wells containing hybrids ^a	Positive in RIA ^b		Antibody secretion ceased	Growth ceased	Discarded ^c	Remainder RIA-positive ^d	
		with antigen	without antigen				with antigen	without antigen
1	13	2	nd	1	1	0	0	0
2	31	6	nd	2	2	1	2	0
7/8	74	11	2	2	3	3	2	1
9/10	45	12	0	2	2	5	3	0

^aat the time of the first screening RIA for that fusion

^bnumbers of wells

^conly highly positive cultures were retained

^dstored in a liquid N₂ freezer

nd = not done

3.4 DISCUSSION

The experiments described in this Chapter established optimal conditions for RIAs useful in the study of fascioliasis. It was clearly shown that RIAs could be used to detect anti-*F. hepatica* antibodies in the sera of 4 mammalian species as well as in supernatants from hybrid cells formed between NSI myeloma cells and spleen cells from mice sensitized to *F. hepatica* antigens.

The optimal concentration of FHS (15 µg/ml) is similar to antigen concentrations used in ELISA tests developed for the detection of antibodies to helminth antigens; 10 µg/ml to 40 µg/ml (Hillyer and Santiago de Weil 1979; Tanaka *et al.* 1979; Craig and Rickard 1980; Iacona *et al.* 1980).

It is likely that a few antigens at high concentrations dictate the optima because that for a pure antigen (about 5 µg/ml to 10 µg/ml; Marier *et al.* 1979; Lunde and Ottesen 1980) is similar to those of crude helminth antigen preparations. The optimum amount of iodinated RAM used in the RIA in this study is higher than most published optima probably because, unlike other studies, the RAM had not been purified by affinity chromatography. Purifying RAM by passage down a column of mouse IgG linked to sepharose removes rabbit IgG molecules that do not bind mouse IgG. This results in a RAM preparation containing far fewer rabbit IgG molecules but all of them, rather than a few, are able to bind mouse IgG.

It can be seen from Tables 3.2 and 3.3 that the use of RAM followed by iodinated protein A in any assay increases the sensitivity of the

assay. Similar results were obtained by Brown *et al.* (1979) who found that assays for monoclonal antibodies to human melanoma cell surface antigens were many times more sensitive if the cells were incubated in RAM before iodinated protein A. It was found that 6×10^4 cpm per well was a suitable amount of iodinated protein A for the RIAs in the present study. Brown *et al.* (1979) used a similar amount of iodinated protein A, namely 5×10^4 cpm per well.

The almost immediate binding of hydrophilic molecules to certain plastics has been reported by Leininger, Cooper, Epstein, Falb and Grode (1966) and Christensen, Johansson and Nielsen (1978) who attempted to overcome the problem of serum proteins binding to plastic tubes after their implantation into humans. Clearly, fluke antigens are no exception in that they rapidly bind PVC. Catt and Treager (1967) found that antigens bind permanently and could not be removed by buffers at pH 2 or 12, and Christensen *et al.* (1978) showed that little antigen is lost during the assay. This property contributes to the reliability of RIA; it can be enhanced by covalently binding antigen to the solid phase (Lehtonen and Viljanen 1980; Rubin, Hardtke and Carr 1980; Rathjen *et al.* 1981).

It was found that incubation of wells with 10% FCS to occupy unbound sites on the PVC was unnecessary, probably because the heterogeneity of the fluke antigen FHS and exposure to FCS in the washing solution combined to ensure that all binding sites became saturated.

Two disadvantages are that chromic chloride cannot bind polysaccharide antigens to RBC and cannot act in the presence of buffer, so if the antigen has been purified the buffer must be removed. In the present study it was hoped that immunization with FHS-coated RBC would increase the probability that IHA could be used to detect hybridomas (see Section 2.1). Thus, the time used in preparing FHS-coated RBC was reduced because most cells used for immunization were those coated for use in IHA tests.

Iodinated RAM was satisfactory in most cases for assaying both mouse and rat antibodies to *F. hepatica* antigens. However, it was inadequate in an assay designed to show that FHS and a preparation of metacercarial secretions have antigens in common (Table 3.3A, serum RA2) as shown by Sandeman and Howell (1981a) and by RIA of MA1 against MFIP (Table 3.2). Therefore, it was concluded that a more powerful RIA was necessary for detection of antibody-secreting rat x mouse hybrid cells. Even though the replacement of RAM by RAR did not make the RIA of rat sera more sensitive, the result was the application of iodinated protein A to assay antibodies of 4 mammalian species. Instead of iodinating RAM, RAR, RAS and protein A to assay sera or hybrid cell culture supernatants that contain mouse, rat, sheep or rabbit antibodies, only protein A need be iodinated. It was found that I^{125} protein A did not bind directly to sheep antibodies in RIA (Table 3.1Di), which is not consistent with the findings of Langone (1980). However, Langone (1980) covalently bound the antigen to agarose beads. The conditions for the binding of protein A to sheep Igs appear to require further study.

Antibodies and protein A completed binding after 90' at room temperature without agitation. This is typical of antigen-antibody reactions. Incubations were extended to 2 h to allow for room temperature fluctuations.

It seems that there is Ig present in serum that binds to PVC directly rather than via antigen (Tables 3.1, 3.2 and 3.4). This was most obvious in assaying rat sera (Table 3.1). Some hybrid cells secreted Ig with the same characteristic (Fig. 3.12). One of these hybrid cell cultures was found to contain mouse IgM (hybridoma MF3; Section 4.3) so perhaps IgM is the source of background binding by sera. The work of Salonen and Vaheri (1979) who showed that another plastic, polystyrene, has a very high affinity for human IgM, supports this suggestion.

Clearly, FHS-coated RBC were as effective as an immunogen as an emulsion of FHH with Freund's adjuvant (Tables 3.2 and 3.4). However, much less antigen was involved. An injection of about 3×10^8 coated RBC used antigen equivalent of 250 μ g protein (see Section 2.2.3) whereas an injection of FHH used 5 mg protein. This result is similar to those obtained by Milgrom, Luszczynski and Dubiski (1956) and Ramshaw and Parish (1977). The former produced antisera to human globulin by injecting it after its attachment to rabbit RBC and the latter studied responses to soluble and MRBC-bound BSA and haemocyanin. Coating of RBC with antigen is time consuming, so applications of this method would probably be restricted to those where little antigen is available or the injection of Freund's complete adjuvant is undesirable.

The sensitivity of the double indirect RIAs could be further increased by purification of RAM, RAR and RAS to eliminate rabbit Ig molecules without specificity for mouse, rat or sheep Ig. However, the effect would probably be small because purification is partly achieved when the rabbit Ig molecules that do not bind non-specifically to the wells and do not have specificity for the target Ig are washed out of the wells before iodinated protein A is added. Another possibility is that the RIAs described above could be converted to ELISAs since enzyme-conjugated protein A has been used in ELISAs as it is in RIAs (Pain and Surolia 1979; Bommeli *et al.* 1980).

Once it was found that the RIA was more sensitive than the IHA test and a gamma counter printout simpler to interpret than a haemagglutination tray, hybrid cells were screened for anti-*F. hepatica* activity by RIA. Figure 3.12 shows that although detection of antibody secreting hybrids was possible, the RIA used was barely powerful enough to perform the task; differences between levels of radioactivity bound by secretors and non-secretors of anti-*F. hepatica* antibody were very small. This demonstrates the need for very sensitive hybrid cell screening assays, especially when using complex mixtures of antigens (see Section 2.4). RIA was able to detect anti-fluke antibody-secreting cells from all 4 mouse x mouse fusions. However, no sheep or rat MCA were detected. Due to its greater sensitivity, the double indirect RIA, rather than a single indirect RIA, was used routinely for retesting antibody-secreting hybrid cells. Unfortunately, it was found that many positive hybrids either became overgrown by non-secreting cells or ceased to proliferate (see Table 3.4). Nevertheless, several hybrid cell cultures were cloned and analysed. These studies are described in Chapter 4.

Chapter 4

CHARACTERIZATION OF HYBRIDOMAS AND MONOCLONAL ANTI-

F. HEPATICA ANTIBODIES

4.1 INTRODUCTION

The derivation of hybrid mouse cells and the detection of those whose secreted products bound to *F. hepatica* antigen in RIA has been considered previously. This Chapter deals with attempts to clone and further characterize these cells. Although their hybrid nature is indicated by their production of antibody directed at fluke antigens, karyotype analysis of cells as well as electrophoresis, isoelectric focussing (IEF) and radial ID of culture medium in which they have grown provide additional evidence. Moreover, karyotype analyses can be used to monitor hybrid cell stability and, if desired, to identify sources of chromosomes. Analyses of cell culture supernatants can indicate the isotypes of MCA and determine the proportions of spleen and myeloma-derived heavy and light chains secreted by hybridomas.

Further analysis of MCA usually involves study of the antigens they bind. Two increasingly common approaches are to localise sites of binding by MCA in tissues and isolate antigens by affinity chromatography or immunoprecipitation (see Sections 1.3.7 and 1.3.8). Both the immunoperoxidase (Capron *et al.* 1975a; Oguz *et al.* 1978; Hillyer 1980; Sandeman 1980) and fluorescent antibody (Thorpe 1965b; Movsesijan and Cuperlovic 1970; Tailliez and Korach 1970b; Rajasekariah and Howell 1978b; Yoshihara *et al.* 1979; Hanna 1980a, b) methods have been used to visualise antibody binding to internal tissues

of *F. hepatica*. For antigen isolation, MCA of the subclasses that bind protein A, such as mouse IgG₁, IgG_{2a} and IgG_{2b} (Ey *et al.* 1978; Goding 1978; McKenzie, Warner and Mitchell 1978), can be bound to protein A sepharose for affinity chromatography (Gersten and Marchalonis 1978; Pearson and Anderson 1980) or to whole, heat-killed, formalised *S. aureus* bacteria (Staph A) for Staph A immunoprecipitation (eg. Handman *et al.* 1980; Potocnjak *et al.* 1980). Alternatively, MCA of any subclass can be immobilized on cyanogen bromide activated sepharose for affinity chromatography (eg. Parham 1979; Sunderland *et al.* 1979; Secher and Burke 1980; Craig *et al.* 1981; Epstein *et al.* 1981).

The species specificity of anti-parasite MCA can be determined in many ways, but is usually achieved by modifying the screening assay (Mitchell *et al.* 1979, 1981; Craig *et al.* 1980; Kim *et al.* 1980). This aspect of MCA analysis is particularly important because the central problem in immunodiagnosis of parasitic infections is crossreactivity (Kagan 1979, 1980). Attempts to produce species specific anti-parasite MCA have had mixed success (see Section 1.3.8).

This Chapter records the cloning and analysis of 4 mouse x mouse hybridomas, 3 of which secreted monoclonal antibody to fluke antigens. One of these hybridomas, MF1, was examined using IEF and polyacrylamide gel electrophoresis (PAGE). In addition, chromosome counts were made. The antigen-binding properties of MF1 monoclonal antibody were studied by (1) RIA, using antigens of various helminths; (2) antigen isolation, using protein A sepharose and Staph A; (3)

antigen localisation by fluorescent and autoradiographic methods. The localisation of monoclonal antibody binding sites within tissues using either radiolabelled MCA or unlabelled anti-helminth MCA has not previously been reported.

4.2 MATERIALS AND METHODS

4.2.1 Cloning Hybrid Cells by Limit Dilution

Hybrid cells were cloned in 96 well flat-bottom Linbro trays (Flow Laboratories). Groups of at least 24 wells received 1 drop from a Pasteur pipette of hybrid cells diluted to between 150 and 3×10^3 cells/ml. Wells also received 3 drops of NSI cells at 10^4 /ml. Since the volume of 1 drop was 30 to 40 μ l, wells contained about 10^3 NSI cells and between 5 and 100 hybrid cells. Feeder cells are commonly used to increase cloning efficiencies (Kennett *et al.* 1980).

The cultures were observed daily until NSI cells covered the well bottoms (about 3 d). Four drops of 10% FCS in double strength HAT medium were then added to kill the NSI cells. Cultures were fed with medium containing HAT until about day 9, HT until about day 15, then normal medium. All media and cells are described in Section 2.2.4.

Supernatants from wells in which hybridoma clones grew were screened for anti-fluke activity by RIA (see below).

Attempts to mimic the feeder effect of NSI cells with conditioned medium, taken from NSI cell cultures 1, 2 or 3 d after inoculating flasks at 5×10^4 cells/ml or 1 d after inoculating flasks with 2×10^5 cells/ml, were unsuccessful.

4.2.2 Radioimmunoassay

Assay method

After the double indirect RIA had been established (Chapter 3) the modifications described below were made in order to screen supernatants of cloned hybridomas rapidly, but without reducing assay sensitivity. Wells of soft PVC trays were coated with 3 drops of antigen from a Pasteur pipette, washed with 1% FCS in 0.9% NaCl followed by distilled water, then stored. The incubation step with FCS (Fig. 3.7, Step 2) was omitted. Supernatants were assayed in duplicate using 1 or 2 drops per well, 2 drops of secondary antibody RAM at 10 $\mu\text{g/ml}$ and 1 drop of radioiodinated protein A containing 6×10^4 cpm. Trays were washed with 0.9% NaCl after each incubation. Other procedures were as described in Section 3.2.4.

Antigens

The preparation of *F. hepatica* antigens FHS and FIP is described in Section 2.2.1. Antigen preparations of adult *S. mansoni*, *Haemonchus contortus*, *Nematospiroides dubius* and *Toxocara canis* and larval *M. corti*, at 20 μg protein/ml, were used to coat soft PVC trays for testing the species specificity of monoclonal antibody MF1 by RIA.

Freeze-dried schistosomes were a gift from Mr John Walker of the Commonwealth Institute of Health, University of Sydney. Adult *H. contortus* were removed from abomasa of experimentally infected sheep and incubated in PBS at 37°C for 4 h in order to allow digestion of blood meals. Antigens of these 2 species were prepared by the methods described in Section 2.2.1.

Soluble somatic antigen preparations of adult *N. dubius* from mice, *T. canis* from dogs at the A.C.T. dog pound and *M. corti* from mice were gifts from Dr S.J. Prowse of the Immunology Department, John Curtin School of Medical Research, ANU and Dr W.L. Nicholas and A.M. Barton of the Zoology Department, ANU, respectively.

4.2.3 *Harvesting MCA*

After cloning, hybridomas were grown in large volumes at similar densities to those of NSI cells (see Section 2.2.4). Cultures of myelomas and hybridomas were centrifuged at 600 g for 7' after they had passed their growth plateaux and had begun to die. Supernatants were stored at -15°C.

Limited success was achieved in separating X63 IgG₁ from FCS in supernatants by 50 or 70% ammonium or sodium sulphate precipitation; significant amounts of FCS persisted (assessed by IEF) and radial ID revealed that over half the IgG₁ was lost. However, purification of X63 IgG₁ on protein A sepharose columns by the method described in Section 3.2.2 removed almost all FCS (as determined by IEF) and retained 30 to 50% of the IgG₁.

4.2.4 *Radial Immunodiffusion*

Concentrated hybridoma supernatants were placed in wells in agar impregnated with anti-mouse IgG₁, IgG_{2a}, IgG_{2b}, IgM or IgA (Meloy radial ID kits) for isotype determinations. Concentrations of

IgG₁ were measured using the supplied standard solution of 849 mg IgG₁ per decilitre (8.49 mg/ml). The standard curve was prepared by plotting IgG₁ concentrations Vs squares of annulus diameters; [diameter of precipitin ring-diameter of well]².

4.2.5 *Isoelectric Focussing*

IEF gels consisted of 10 ml 29.1% acrylamide (BDH), 10 ml 0.9% N N'-methylenebisacrylamide (Eastman), 0.5 ml 0.01% riboflavin (Calbiochem), 7.5 g sucrose, 3 ml ampholine (pH 3.5 to 10, LKB) and distilled water to 60 ml. Gels were run on an LKB Multiphor apparatus at a constant power of 30 W with 30' for equilibration and 90' for protein separation. Electrode solutions were 1 M NaOH (cathode) and 1 M H₃PO₄ (anode). Gels were fixed in 5% sulphosalicylic acid, 10% trichloroacetic acid in 10% acetic acid, stained with 0.2% Coomassie blue in 35% ethanol/10% acetic acid and destained in 35% ethanol/10% acetic acid.

4.2.6 *Polyacrylamide Gel Electrophoresis of Secreted Cell Products*

Ten to 17% polyacrylamide gradient gels and autofluorographs were prepared by Dr T.J. Higgins of the Division of Plant Industry, CSIRO, according to the methods of Higgins, Goodwin and Whitfield (1976) and Higgins and Spencer (1977).

The secreted products of X63, NSI and MF1 cells were biosynthetically radiolabelled by incubation with C¹⁴-leucine (Radiochemical Centre, Amersham). Cells were harvested at the growth plateau (about 4 x 10⁵/ml), washed in leucine, methionine and glutamine free DMM (Flow) reconstituted with 0.003% methionine

(Sigma) and 0.058% glutamine (Sigma). Cells were preincubated at 37°C for 15' in 2 ml leucine free DMM then 5×10^6 cells were suspended in 1 ml of this medium containing 100 μ l C^{14} -leucine (50 μ Ci/ml; specific activity 2.48 mCi/ μ g) and cultured overnight. After about 20 h the cells were removed by centrifugation at 1,000 g for 10'.

4.2.7 *Antigen Localisation*

Radiolabelled and unlabelled cell supernatants were used in attempts to localise antigen in frozen sections of adult flukes obtained from experimentally infected sheep. Autoradiographic and fluorescent methods were employed.

Autoradiography

Flukes were cut into pieces and frozen with solid CO_2 either soon after removal from livers or after fixation overnight in 10% formalin in PBS. Cryostat sections (10 μ m) of fresh tissue were post-fixed for 10' in either 10% formalin in PBS, cold 95% ethanol or cold acetone, then washed with 0.9% NaCl. Sections were then treated with 1% FCS in PBS for 30' then 10 μ l C^{14} -labelled X63 or MF1 supernatant for 1 h at room temperature.

After washing with 0.9% NaCl slides were dried and coated with Kodak nuclear track emulsion NTB3 and stored dry at 4°C for 2 to 7 d. Autoradiographs were developed in Kodak D19, fixed, then stained with haematoxylin and eosin. Sections were slowly dehydrated by immersion in 95% ethanol for 10' then 100% ethanol

for 30'. This was followed by treatment with 20% phenol in xylene for 30', 2 changes of xylene for 10' each then a xylene/Canada balsam 1:1 mixture for 1 h before mounting in Canada balsam.

Fluorescent Antibody Method

The first fluorescent antibody method employed was similar to that used by Rajasekariah and Howell (1978b). However, although the method gave specific fluorescence with mouse antisera it failed to do so with monoclonal antibody MF1. Therefore, an adaptation of the method used by Barnstable (1980) for localising MCA in rat retina was adopted.

Flukes were cut into about 5 pieces for fixation for 1 h at 4°C in 1% paraformaldehyde, 0.1% glutaraldehyde in 0.1 M pH 7.6 phosphate buffer (PB) then soaked in 30% sucrose overnight. Frozen sections were placed on slides subbed with 0.5% gelatin. Following treatment with 5% BSA in PB for 1 h then 4-fold concentrated MF1 or X63 supernatants for about 20 h at 4°C in a humid chamber, sections were washed with 0.9% NaCl. They were then incubated for 1 h at room temperature with fluorescein-conjugated rabbit IgG anti-mouse IgG (Wellcome) or normal rabbit serum diluted 1 in 12 in 5% BSA in PB, washed and mounted in glycerol/PBS (1:1). Sections were examined using a Leitz ultraviolet photomicroscope using a BG12 exciter filter, as well as with phase contrast optics. Some sections were stained with haematoxylin and eosin in order to identify structures more readily.

4.2.8 *Antigen Isolation*

Several attempts were made to use the MF1 antibody to remove its corresponding antigen from preparations of soluble somatic fluke antigens. All relied on the affinity of mouse IgG, the isotype of both the MF1 and X63 Igs, for protein A and resolution of the isolated antigen by SDS-PAGE. The first method involved reacting protein A sepharose with MF1 antibody then FHS; the second was similar but the MF1 antibody was crosslinked to the protein A sepharose; the third was similar to the first but used Staph A instead of protein A sepharose.

SDS-PAGE and, when necessary, fluorography, were performed by D.O. Irving of the Zoology Department, ANU, according to the methods described by Irving and Howell (1982a). Briefly, proteins were precipitated in acetone then reduced by exposure to 2-mercaptoethanol, SDS and heat. Vertical slab gradient gels having a 5% stacking gel were run at 30 mA for about 3 h and stained with 0.2% Coomassie blue.

Protein A Sepharose Method

In the first method, either 0.2 ml rabbit anti-FHH (Section 2.2.2) or 600 µg MF1 antibody in 0.4 ml FCS were incubated with 100 µl protein A sepharose in 400 µl 0.1 M pH 8.0 phosphate buffer using the reagents listed in Section 3.2.2. The sepharose was washed with 0.1 M pH 8 phosphate buffer by decantation before 20 mg FHS in 1 ml 0.1 M pH 8 phosphate buffer was added. The unbound FHS was removed

30' later, precipitated in acetone and prepared for SDS-PAGE as described above. The sepharose was washed in 0.1 M pH 8 phosphate buffer and treated with 0.5 ml 0.1 M pH 3 citrate buffer for 20' to elute bound antibody and antigen. The supernatant was prepared for SDS-PAGE as described above.

Protein A Sepharose Crosslinking Method

A method devised by Gersten and Marchalonis (1978) for crosslinking antibody to protein A sepharose in order to elute bound antigen without eluting antibody was adapted to the current project. Protein A sepharose beads were washed with P'BS (0.02 M phosphate, 0.85% NaCl, pH 7.0). 200 μ l of beads were then incubated with 500 μ g MF1 antibody in 1 ml P'BS for 30' at room temperature, followed by washing 5 times with 1.5 ml P'BS and twice with 1.5 ml 0.1 M pH 8 borate buffer. Treatment with the crosslinker, dimethylsuberimidate dihydrochloride (DMS; Pierce) was for 30' at room temperature using 5 mg DMS in 1.5 ml 0.1 M pH 8 borate buffer. This was followed by a 30' incubation of the beads with 1.5 ml P'BS, 3 washes with P'BS then incubation overnight at 4°C with 1.5 ml CBS (0.1 M citrate, 0.85% NaCl, pH 3.0). After washing with P'BS the beads were incubated for 30' at room temperature in 1.5 ml P'BS containing 20 mg FHS and 1 mg BSA then washed 5 times with P'BS. Four washes with CBS were used to elute any bound antigen. The IgG₁ contents of samples from the incubations of beads with CBS were measured to ensure that antibody had bound to the beads.

Staph A Immunoprecipitation

The preparation of and attempts to use Staph A, provided by Dr C.R. Parish of the Microbiology Department, John Curtin School of Medical Research, ANU, for isolating fluke antigens were performed by D.O. Irving of the Zoology Department, ANU, using the methods described by Irving and Howell (1982b). These methods were essentially those of Kessler (1975, 1976) except that the Staph A was preincubated with fluke antigens to reduce nonspecific binding and Triton-X 100 was used instead of Nonidet P-40.

For this experiment somatic antigens of 3 weeks old flukes, grown in and collected from mice according to the methods described by Howell and Sandeman (1979), were biosynthetically labelled with C^{14} -leucine by Dr M.J. Howell using the method described by Irving and Howell (1982b).

4.3 RESULTS

Table 3.4 (Chapter 3) shows that a number of hybrid cells were found to secrete anti-fluke antibody but only some of these were cloned. It was found that a 5% CO_2 gas phase was essential for the survival of cells in 96 well trays. Supernatants from NSI cultures did not appear to influence cloning efficiencies (0.1 to 0.5%), so NSI cells were used as feeder cultures to increase hybridoma cloning efficiencies to between 0.4 and 4%.

Eight hybridomas were cloned, 4 of which were stored without further analysis. Mouse anti-*F. hepatica* hybridoma 1 (MF1), from

fusion 9/10, was analysed in detail because it grew more readily and produced greater levels of binding in RIA than MF2 and 4. Moreover, MF1 antibody could readily be used in protein A sepharose columns because its isotype was IgG₁. MF2, also from fusion 9/10, was found by radial ID to secrete IgM, which does not produce Ig bands following IEF in polyacrylamide gels (not shown). However, this hybridoma was shown to secrete anti-fluke antibody; it did not bind directly to PVC in RIA. MF3, from fusion 7/8, secreted IgM and bound PVC directly in RIA. MF4, from fusion 2, was IgG₁ but weakly positive in RIA compared with MF1. These 4 hybridomas were recloned up to 3 times until 96 to 100% of the clones were positive in RIA. Attempts to reveal the presence of anti-fluke antibody in 10 and 20-fold concentrated MF1, MF2 and MF4 supernatants by IHA or ID were unsuccessful.

MF1 cells were found to contain between 70 and 82 chromosomes 3 months postfusion and after a further 5 months *in vitro*. During continuous culture of MF1 for 9 months the FHS-binding capacity of its fresh culture supernatants in RIA remained constant. However, FHS binding by freeze/thawed supernatants diminished to negligible levels after about the third thaw.

Supernatants from MF1 and X63 cultures contained up to 60 µg IgG₁/ml. IEF of 5-fold concentrated MF1 and X63 supernatants in a pH 3.5 to 10 gradient showed that the 2 Igs migrated to different positions in the gel; that of MF1 IgG₁ was more anodic (Fig. 4.1). Each IgG₁ produced several bands, presumably due to small charge differences between subsets of each secreted Ig. By making comparisons with focussed MF2 and NSI supernatants (not shown), some of the bands shown in Fig. 4.1 were judged to be FCS-derived and others Ig-derived.

The C^{14} -labelled secreted products of X63, MF1 and NSI cells were compared by PAGE. A far greater amount of radiolabelled material was produced by MF1 than X63 cells in 3 incubations with C^{14} -leucine. Therefore, 10 times as many TCA-precipitable cpm of X63 as MF1 products needed to be loaded onto the gels to obtain the result shown in Fig. 4.2 (T.J. Higgins, pers. comm.). The non-Ig products of NSI, X63 and MF1 cells appear to be similar (Fig. 4.2, tracks 1, 2 and 3) but the electrophoretic mobilities of X63 and MF1 light chains differed (Fig. 4.2, tracks 2, 3, 6 and 7). Secretion by MF1 cells of MOPC 21 light chains in addition to spleen cell derived light chains, as would be expected (see Section 1.3.1), was not detected (Fig. 4.2, track 7). The SDS-PAGE also showed that, of the many secreted products of myeloma cells, only the Ig bound to protein A sepharose (Fig. 4.2, tracks 4 and 5).

RIAs performed in triplicate to determine the specificity of the MF1 antibody revealed that it did not bind FIP or FCS but bound soluble somatic antigens of adult flukes, schistosomes, *H. contortus*, *N. dubius* and *T. canis* and larval *M. corti* (Table 4.1). It was found to be necessary to run individual X63 supernatant controls for each helminth species because control levels of binding were up to 6 times higher using *M. corti* and *S. mansoni* antigens than the other antigens.

Attempts to use autoradiography to localise MF1 antibody binding in frozen sections of adult flukes were unsuccessful. Silver grain development over the tissue sections was no greater with MF1

than with X63 radiolabelled supernatants. The protocol was varied with respect to fixation, antibody incubation times, radiolabelled supernatant concentrations and exposure times.

Similarly, many variations on the initial fluorescent antibody method were employed in unsuccessful attempts to localise MF1 antibody binding to fluke tissues. However, adapting the monoclonal antibody localisation method of Barnstable (1980) to the problem gave localised, specific fluorescence (Figs. 4.3, 4.4 and 4.5). Specific binding by MF1 antibody was restricted to (1) a narrow band, probably the basal membrane of the tegument, immediately external to the circular muscles (Fig. 4.3); (2) testes tubules (Figs. 4.4 and 4.5) and (3) an area underlying the uterine (not shown) and caecal cells (Fig. 4.5). In some flukes MF1 antibody bound to the epithelial cells lining the caeca and testes tubules as well as below these cells (Fig. 4.5). MF1 antibody did not bind vitellaria, caecal contents, parenchymal cells, testicular cells, sperm, eggs, tegumental cells, the tegumental epithelium or its tonofibrils or muscles of the tegument, uterus, sperm ducts, caeca or suckers (Figs. 4.3, 4.4 and 4.5). The cirrus, ootype, ovary, cerebral ganglia and main excretory ducts were not present in the sections incubated with MF1 antibody.

Normal mouse serum and X63 supernatants produced low levels of background fluorescence throughout the sections, whereas mouse anti-fluke serum MA1 bound to most structures but especially the caeca, oral and ventral suckers and tegument.

This fluorescent antibody method avoided the autofluorescence often associated with the tonofibrils of the tegument, which are concentrated in the spines, and the caeca and vitellaria (not shown; Thorpe 1965b). However, autofluorescence of egg shells occurred.

By radial ID it was found that about 30 to 50% of the IgG₁ in MF1 and X63 supernatants, loaded in subsaturating amounts, bound to protein A sepharose. All the IgG₁, plus detectable amounts of up to 4 other molecules (Fig. 4.6, tracks 4 and 5) were eluted by buffers of pH 3 to 4.5. The 4 non-Ig molecules, one of which appeared to be BSA (compare tracks 1, 2 and 4, Fig. 4.6), may have been components of the cell culture medium. They were also present as unlabelled constituents in preparations of radiolabelled X63 and MF1 products (Coomassie blue staining of the gel shown in Fig. 4.2, tracks 4 and 5, is not shown).

Attempts to use protein A sepharose to isolate the fluke antigen(s) to which MF1 antibody (Fig. 4.6) or rabbit anti-FHH antibodies (not shown) bound failed; binding by fluke antigen to MF1 antibody-protein A sepharose was not detected using SDS-PAGE designed to visualise either removal of an antigen from FHS (Fig. 4.6, tracks 2 and 3) or elution of an antigen bound to antibody (Fig. 4.6, track 5). An alternative method, in which Staph A was preabsorbed with FHS then incubated with a mixture of MF1 antibody and C¹⁴-labelled soluble somatic antigens, was also unsuccessful; the same radiolabelled fluke antigens coeluted with both the MF1 and X63 antibodies (Fig. 4.7, tracks 2 and 3 - the photograph failed to reproduce all the bands visible on the fluorograph).

Table 4.1 SPECIES SPECIFICITY TEST OF MONOCLONAL ANTIBODY MF1
BY RADIOIMMUNOASSAY.

Antigen	X63 supernatant ^a	MF1 supernatant ^a	MF1 binding- multiple of control
<i>F. hepatica</i>			
FHS	230	1350	5.9
FIP	140	190	1.4
<i>S. mansoni</i>	600	2650	4.4
<i>H. contortus</i>	150	3300	22.0
<i>N. dubius</i>	110	1750	15.9
<i>T. canis</i>	100	400	4.0
<i>M. corti</i>	550	1700	3.1
FCS	90	110	1.2

^acpm bound by fresh supernatants in double
indirect RIA

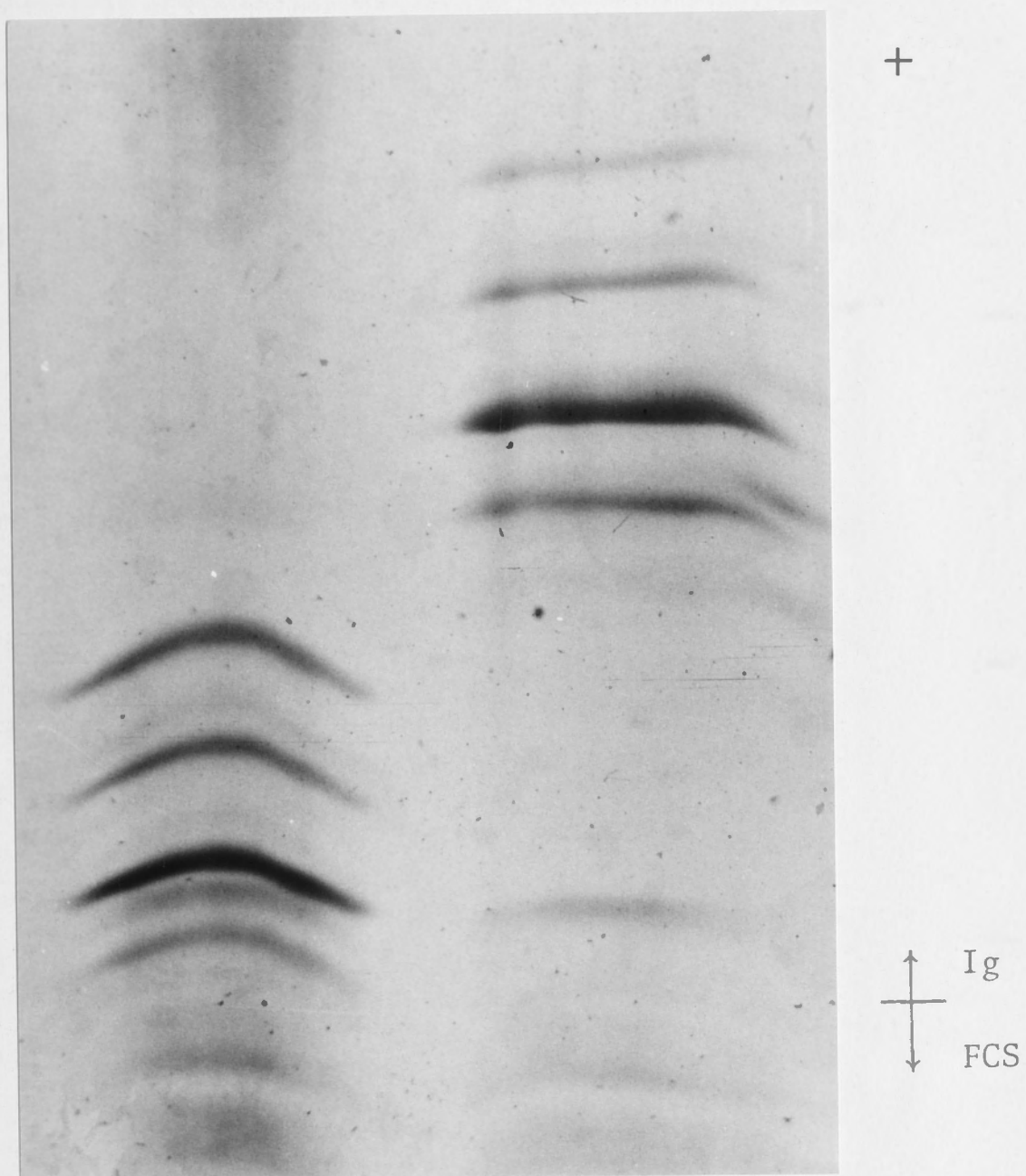


Figure 4.1 Isoelectric focussing in 10% polyacrylamide of X63 (left) and MF1 (right) antibodies. Only the part of the gel that contained Ig molecules is shown. Coomassie blue stain.

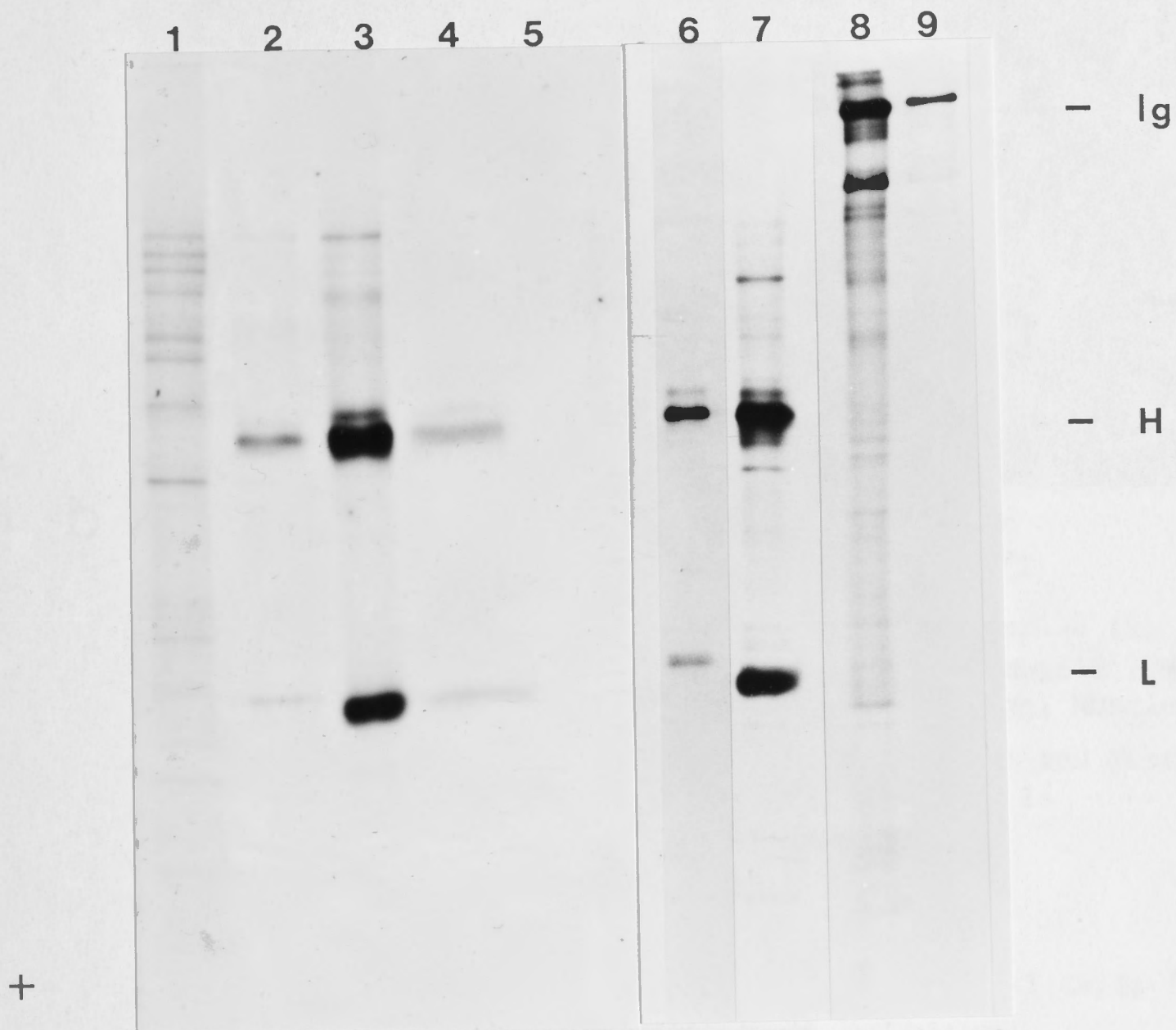
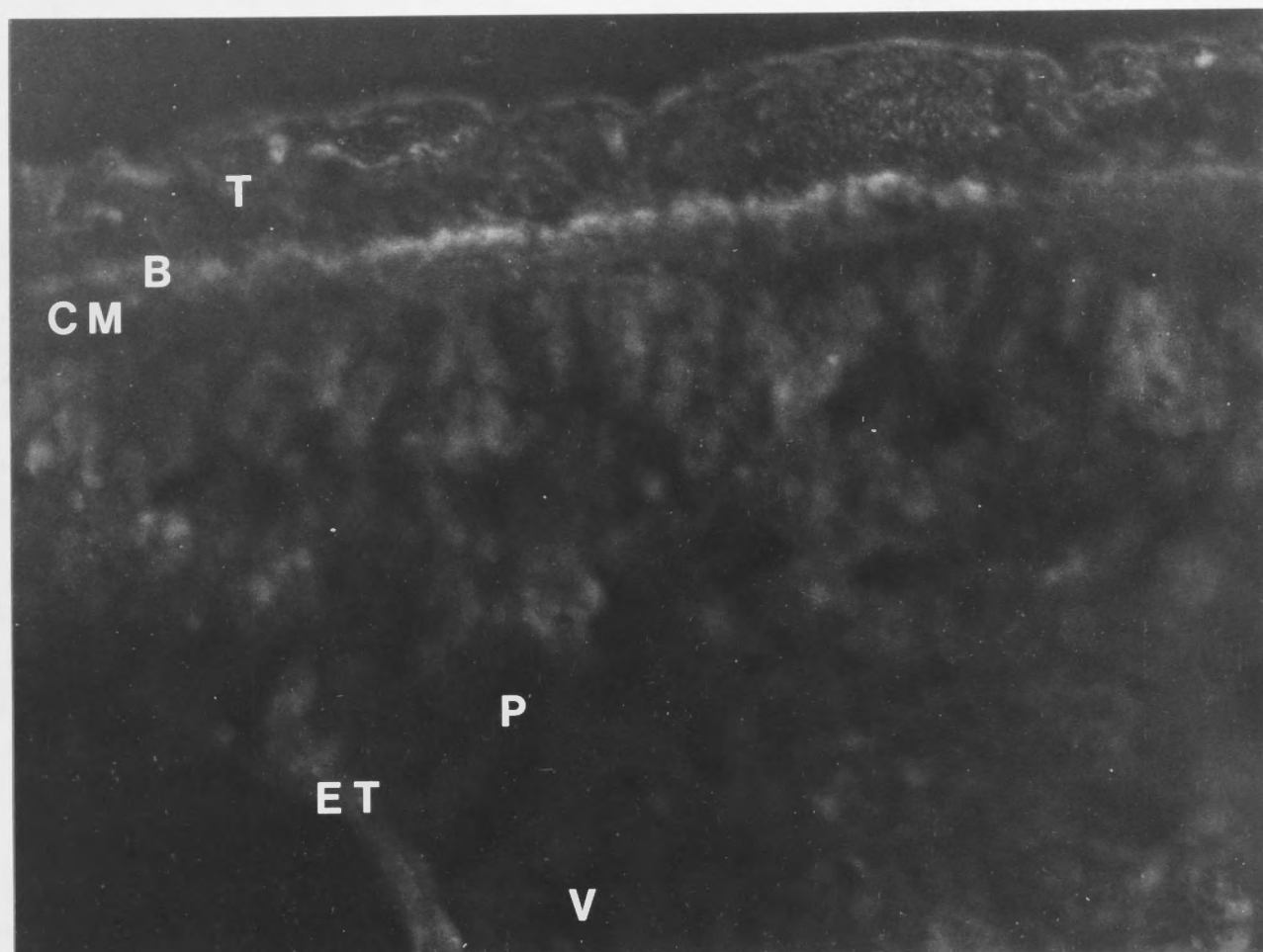


Figure 4.2 Fluorographs of PAGE of C^{14} -labelled secreted cell products.

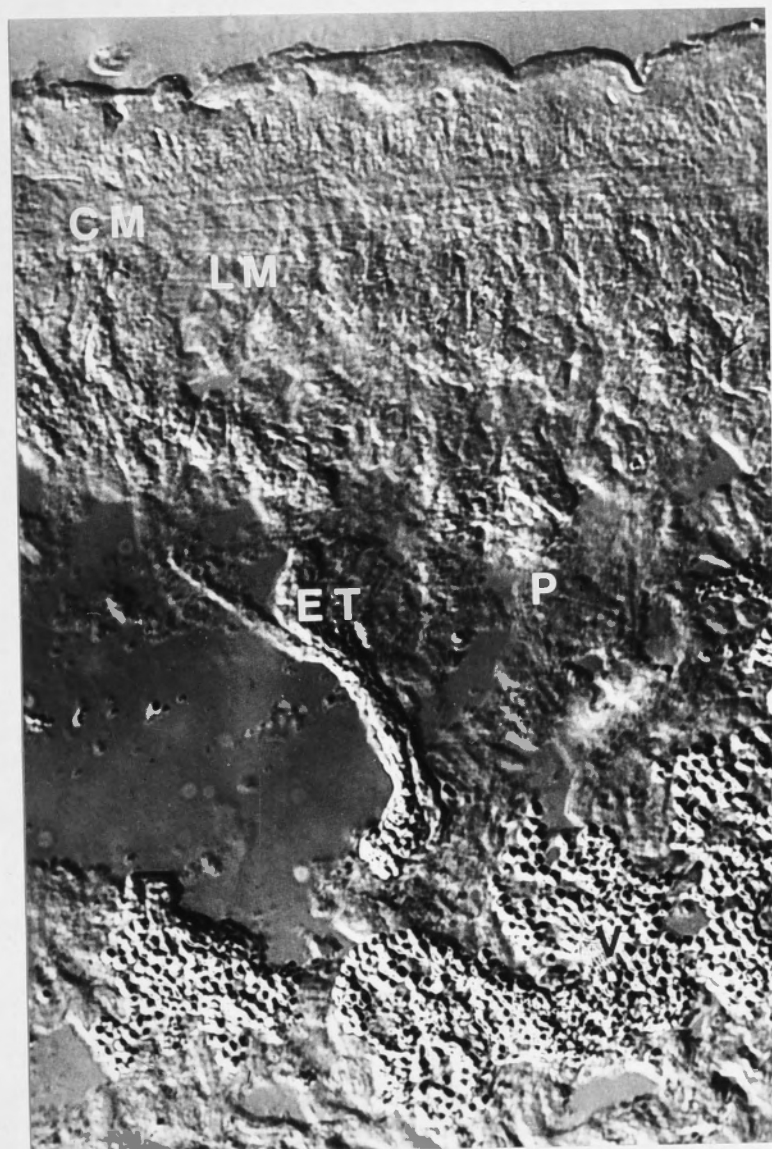
SDS-reduced gels: NSI products (1) and those that bound to protein A sepharose (5); X63 products (2 and 6); MF1 products (3 and 7) and those that bound to protein A sepharose (4).

Undreduced gel: MF1 (8) and X63 (9) secreted products. The mobilities of intact Ig and Ig heavy (H) and light (L) chains are shown.

a



b



Tegument (T)
 Basal Membrane (B)
 Circular Muscle (CM)
 Longitudinal Muscle (LM)
 Tegumental and Muscle
 cells

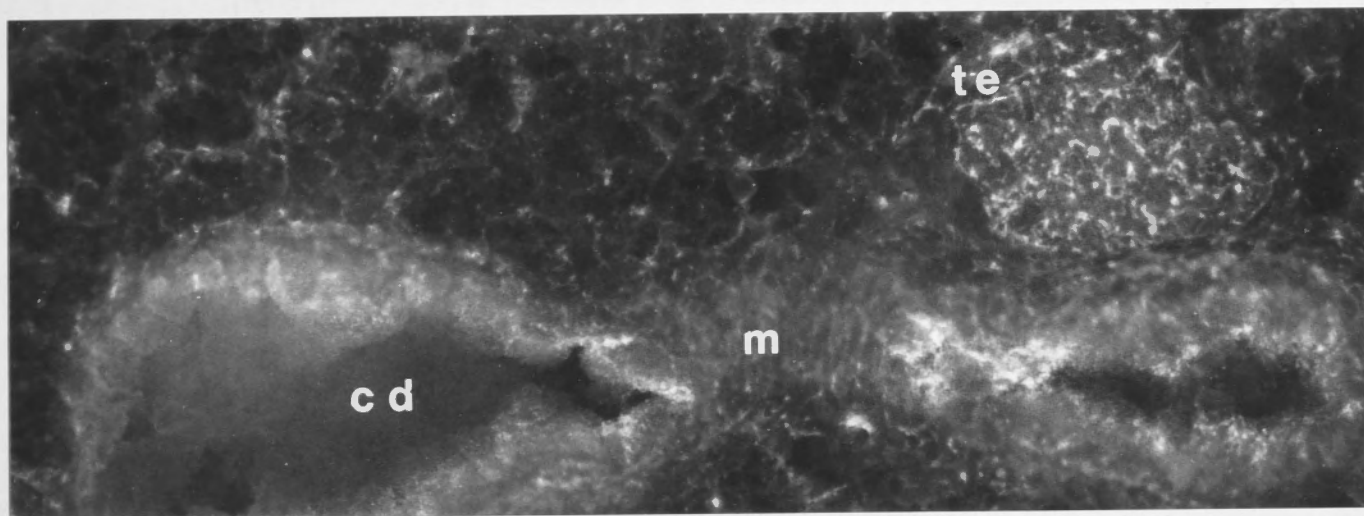
 Parenchymal Cells (P)

 Excretory Tubule (ET)

 Vitellaria (V)

Figure 4.3 Antigen localisation. (A) Binding by MF1 antibody to the basal membrane of the tegument shown by a fluorescent antibody method (x 300). (B) Phase contrast photomicrograph of the same transverse section as in (A) (x 250).

a



b

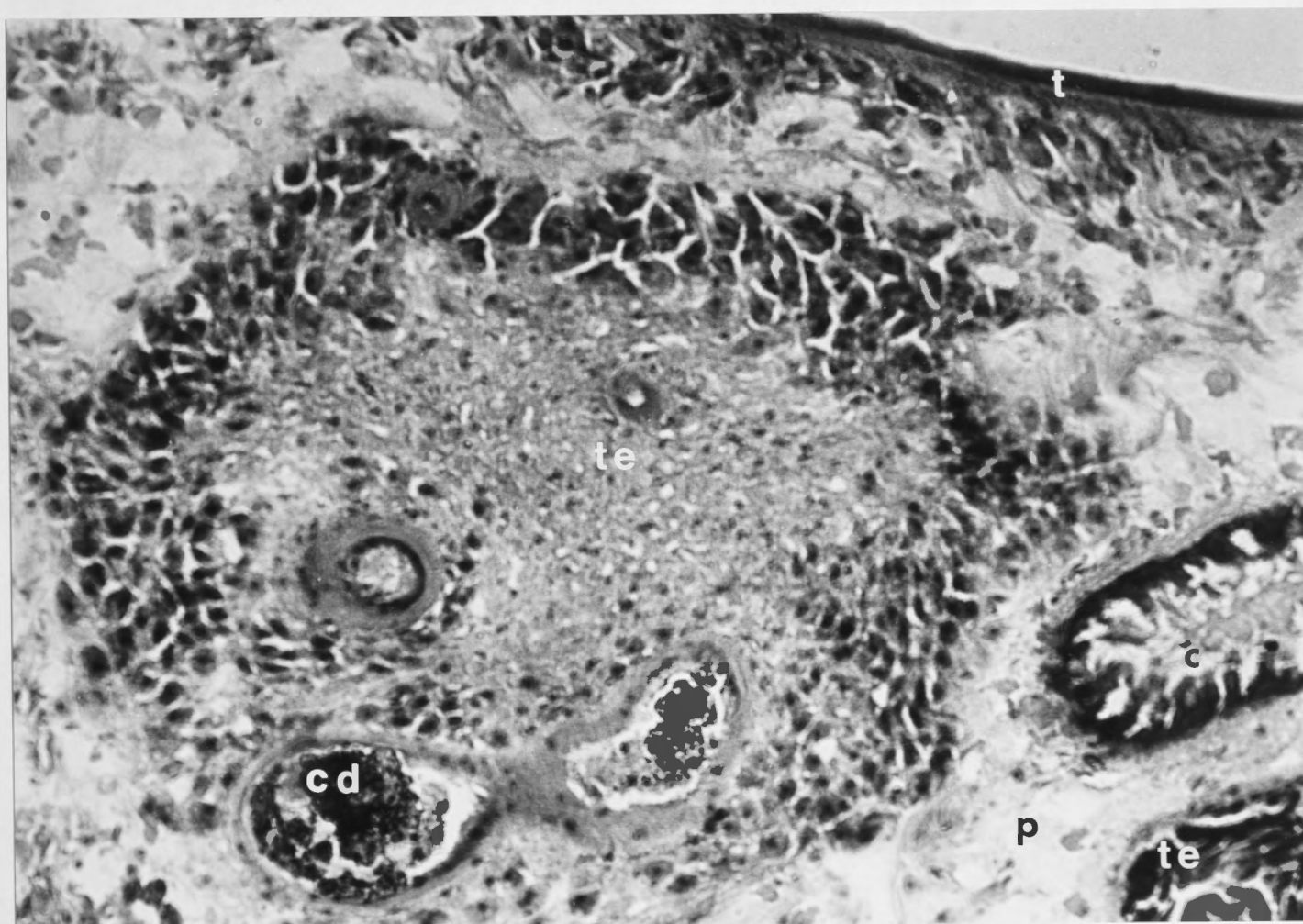


Figure 4.4 Antigen localisation. (A) Specific fluorescence due to MF1 antibody binding to testes (TE) tubules and walls but not muscles (M) of a collecting duct (CD) (x 300). (B) Haematoxylin and eosin staining of a similar transverse section of the same fluke showing testes, caecum (C) and tegument (T) (x 120).

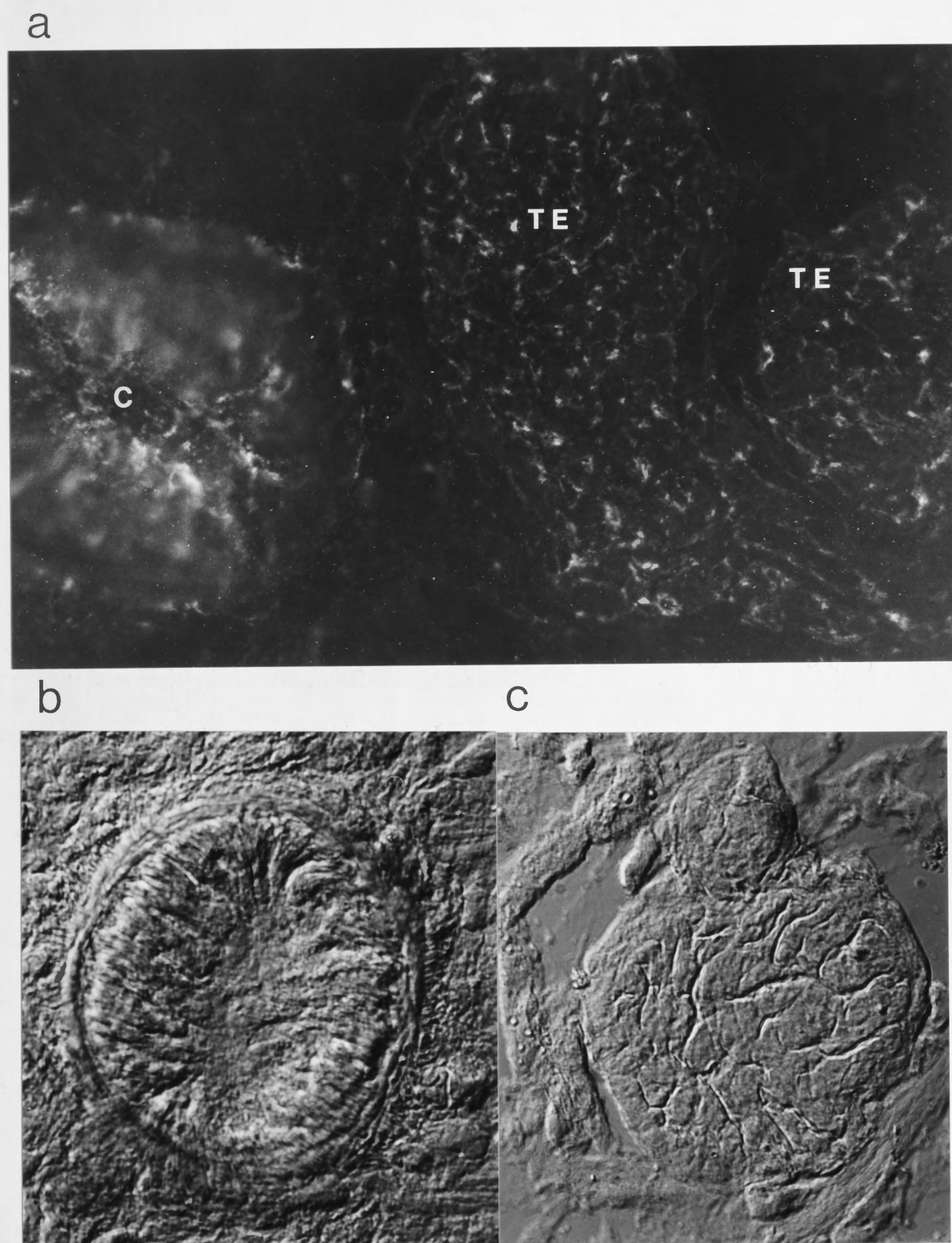


Figure 4.5 Antigen localisation. Specific fluorescence due to MF1 antibody binding to caecal cells and testes tubules (A; x 500). Phase contrast photomicrographs of similar transverse sections of caeca (B; x 500) and testes (C; x 480).

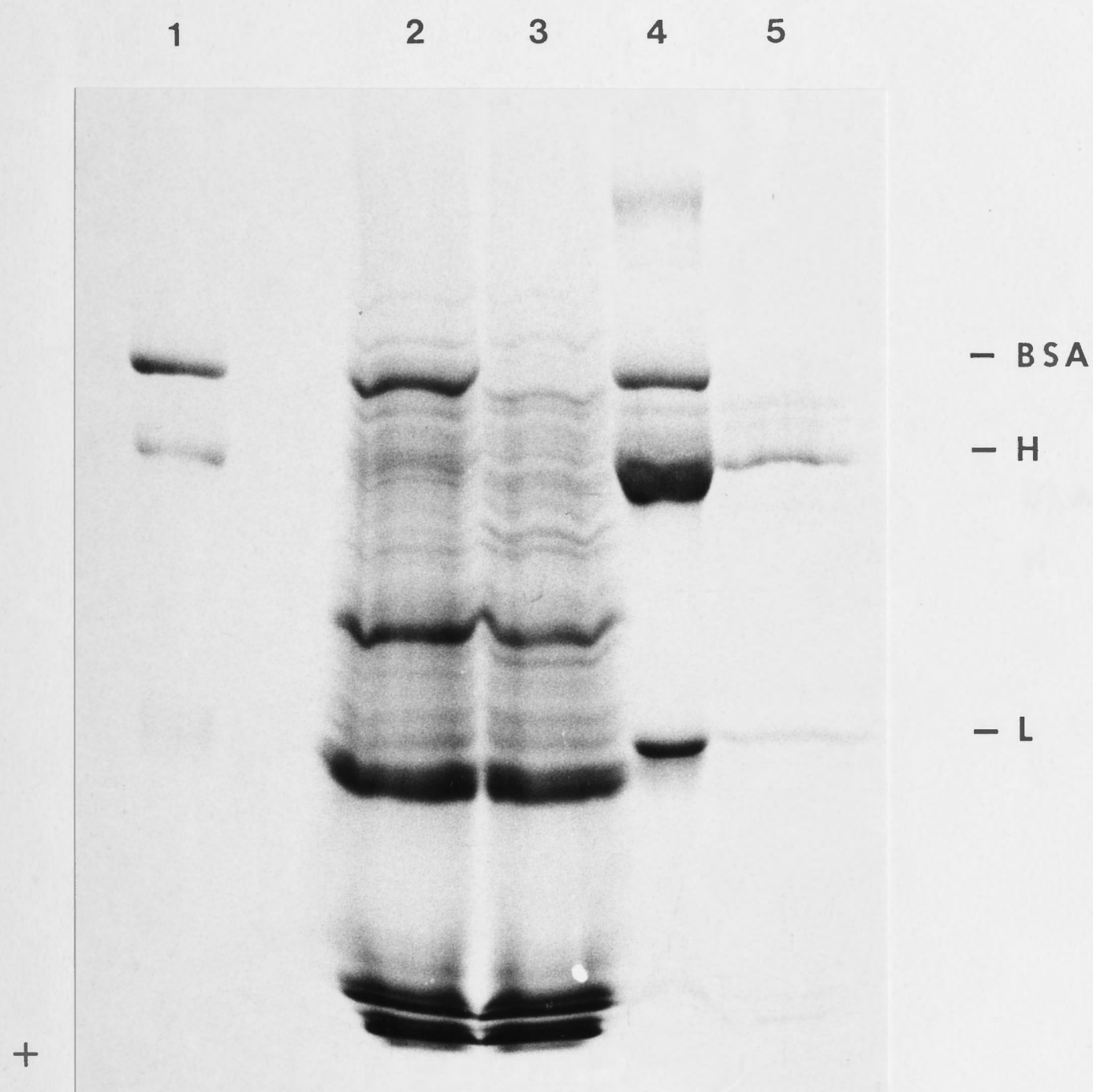


Figure 4.6 Attempted antigen isolation. SDS-polyacrylamide 7 to 15% gradient gel, stained with Coomassie blue, of sheep IgG and BSA (1); FHS that did not bind to MF1 IgG₁-protein A sepharose (2); FHS (3); MF1 IgG₁ after purification on a protein A sepharose column (4); pH 3 eluate from protein A sepharose after incubation at pH 8 with MF1 supernatant then FHS (5). The mobilities of BSA, Ig heavy chains (H) and Ig light chains (L) are shown.



Figure 4.7 Attempted antigen isolation. Fluorograph of SDS-polyacrylamide 10 to 15% gradient gel of soluble somatic components of *F. hepatica* biosynthetically labelled with C^{14} -leucine (1) and those eluted from Staph A preincubated with X63 IgG₁ (2) and MF1 IgG₁ (3).

4.4 DISCUSSION

Four mouse x mouse hybrid cell cultures previously shown to produce anti-*F. hepatica* monoclonal antibody (Section 3.3) were cloned. One of these, the MF1 hybridoma, deemed most suitable for study due to its stability and antigen-binding properties, was shown to secrete IgG₁ that differed from that of the X63 myeloma, thus demonstrating the hybrid nature of MF1. MF1 supernatants were found to bind to soluble somatic antigens of several helminths in RIA and to the testes tubules and structures underlying the uterus, caeca, sperm collecting ducts and tegument of adult flukes. Although NSI was the myeloma parent of MF1, it was considered that X63 is a more suitable control in most experiments because it, like MF1 but unlike NSI, secretes Ig.

The stability of the MF1 hybridoma was confirmed by its persistent secretion of antibody during 18 months of culture interrupted by 5 months storage under liquid N₂. Unfortunately, antigen-binding activity was rapidly lost if the antibody was repeatedly frozen and thawed, a problem that is commonly encountered with MCA (Goding 1980).

The ineffectiveness of ID and IHA for screening anti-fluke MCA (see Section 2.4) was confirmed while the RIA proved its value for monitoring antibody production and screening clones. It appears that only some mouse IgM produces false positives in RIA (see Section 3.4) because although both MF2 and MF3 antibodies were IgM, only the MF3 antibody bound PVC directly.

Further evidence of the hybrid nature of MF1 was the fact that it possessed between 70 and 82 chromosomes, in contrast to the X63 line which has 61 to 66 chromosomes. X63 and NSI cells probably possess similar numbers of chromosomes.

The MF1 and X63 lines were shown to secrete different Igs by IEF and fluorography/electrophoresis of cell supernatants (Figs. 4.1 and 4.2). IEF produces several bands for each Ig (Fig. 4.1; Köhler and Milstein 1976; Köhler *et al.* 1977) or heavy or light chain (Köhler *et al.* 1977) which indicates that these molecules vary with respect to charge. These small differences in charge may be due to (1) differences (perhaps one codon) between gene copies; (2) variability in post-transcriptional and/or post-translational processing; (3) the minimal nutritional content of the culture medium which may cause shortages of some amino acids and/or (4) prolonged exposure to the potentially damaging culture conditions. Another indication of heterogeneity in these monoclonal Igs is that usually less than half bound to protein A sepharose, which suggests that some of the molecules have defects in their protein A binding sites.

The apparent lack of NSI-derived MOPC 21 κ chain secretion by MF1 (Fig. 4.2) is advantageous because it means that all the Ig molecules secreted by MF1 should bind FHS (see Section 1.3.1). Springer (1980) has shown that the level of κ chain secretion by NSI-parented hybridomas can vary from nothing up to that of X63.

It was shown that MF1 antibody does not bind fluke incubation products (FIP) and is not specific for *F. hepatica* (Table 4.1). Antigen preparations of another trematode (*S. mansoni*), a cestode (*M. corti*) and 3 nematodes (*H. contortus*, *N. dubius* and *T. canis*) bound to this monoclonal antibody. This does not preclude the use of MF1 for developing an immunodiagnostic reagent. Possibly, removal of the MF1-binding, crossreactive antigen from FHS would yield a mixture of antigens having immunodiagnostic potential. However, this would be necessary only if the crossreactive antigen elicits a humoral response during infection, which seems likely upon consideration of the antigen localisation experiments (see below). It is unlikely that the MF1 antibody binds to a host-like antigen common to the parasite antigen preparations because these parasites were taken from different host species (sheep, dogs and mice). Furthermore, MF1 did not bind to FCS, and there is much greater crossreactivity between helminths than between helminths and their hosts (see Section 1.2.5; Capron *et al.* 1968).

The level of binding by MF1 antibody to *F. hepatica* and *T. canis* antigens appeared to be lower than to other helminth antigens (Table 4.1). However, the former antigens had been stored at -15°C for more than a year and the *T. canis* antigen preparation had been frozen and thawed several times. These antigens could, therefore, have been damaged. The other antigens were used within a short time of their preparation. The finding that X63 antibody bound to larval *M. corti* and adult *S. mansoni* antigens (Table 4.1) suggests that these

parasites possess receptors for Ig but that *F. hepatica* and the 3 nematodes do not. Experiments by Hanna (1980b) and Howard *et al.* (1980) indicate that flukes do not possess Fc receptors as a means of evading immune responses (see Section 1.2.3). In contrast, the schistosome tegument has been shown to possess Fc receptors (Torpier *et al.* 1979; Santoro *et al.* 1980a; Tarleton and Kemp 1981) which would be expected to bind mouse IgG₁, and perhaps RAM. Tetrathyridia of *M. corti* are known to be covered by host Ig *in vivo* (Mitchell *et al.* 1977; Washington, Barton, Nicholas and Stewart 1982) but the proportions of this Ig that bind to worms cytophilically and opsonically have not been determined.

The ability of the MF1 IgG₁ to bind to protein A could, if desired in the future, be exploited to simultaneously purify and radioiodinate it on a protein A sepharose column using the method described by Tsu and Herzenberg (1980). Other implications of the affinity of mouse IgG₁ for protein A were exploited in the present study in efforts to purify the MF1 IgG₁ and isolate the antigen to which it binds. Reports of parasite antigen isolation using MCA attached to Staph A (Handman *et al.* 1980; Potocnjak *et al.* 1980) or sepharose 4B (Pearson and Anderson 1980; Craig *et al.* 1981; Epstein *et al.* 1981) indicate that a reason for the failure to isolate fluke antigens using protein A immunoadsorbents in the present study may be that the methods employed were unable to detect small amounts of isolated, C¹⁴-labelled fluke antigens or used insufficient quantities of biologically active material. Published work has used either high specific activity

isotopes (S^{35} or I^{125}) to label antigens or large amounts of monoclonal antibody and unlabelled antigens.

Irving and Howell (1982b) found that C^{14} -leucine and S^{35} -methionine were equally suitable for the biosynthetic labelling of fluke antigens for antigen isolation experiments using antisera. Conceivably, these experiments would have detected only the antigens that either became strongly labelled or were present at high concentrations. If the antigen bound by MF1 antibody is not in this category, its detection might depend upon its radioiodination. The feasibility of radioiodinating internal antigens of helminths is unknown. However, intact juvenile liver flukes (Howard *et al.* 1980), schistosomes (Hayunga, Murrell, Taylor and Vannier 1979; Snary *et al.* 1980) and solubilized schistosome tegumental antigens (Hayunga *et al.* 1979) have been radioiodinated. The Bolton-Hunter method for radioiodinating antigens would probably be the most suitable to use in further attempts to isolate the antigen that binds to MF1 antibody because it labels any free amino groups (Hayunga *et al.* 1979). Alternatively, it may be that the antigen to which MF1 antibody binds contains no amino acids and would not be revealed by I^{125} , C^{14} -leucine or S^{35} -methionine labelling or Coomassie blue staining. Flukes are known to contain non-protein antigens (see Section 1.2.5), so other labelling and staining methods should be attempted. Another possibility is that the antibodies used lost their antigen binding activity before or during the antigen isolation experiments.

The existence of a fluorescent antibody method for localising the binding sites of MCA in fluke tissues (Figs. 4.3, 4.4 and 4.5) means that it could be modified so that autoradiographic, immunoperoxidase or ferritin methods of visualising binding sites could be used. These would have the advantage of providing more permanent preparations than the fluorescent method. The method described here for preparing flukes for antigen localisation produces low background fluorescence and few autoradiographic artefacts. Nevertheless, background levels of silver grain development could probably have been reduced by removing free C^{14} -leucine by gel filtration or affinity chromatography. If MCA to antigen in a particular tissue such as the tegument were desired, a localisation method such as fluorescence may be more suitable than a RIA as a screening assay.

The pattern of binding by the MF1 antibody - to structures associated with the testes, caeca, uterus and tegument - does not necessarily indicate that the antigen bound by this monoclonal antibody is functional. However, the discovery that MF1 antibody bound to caecal cells and near the worm surface suggests the possibility that it could be used to damage worms. This suggestion could be tested by electron microscopy of worms after *in vitro* incubation with monoclonal antibody and other immune effectors such as complement and peritoneal cells, or by placing monoclonal antibody and flukes in the peritoneal cavities of naive rats. The latter immunoassay, which is rapid and simple to perform (Hughes *et al.* 1981), relies on the finding by Goose and Macgregor (1973), Hughes *et al.* (1976b, 1977) and Bennett *et al.* (1980) that adult flukes transferred to the peritoneum of an immune rat die within 12 h.

The pattern of binding by MF1 antibody to sections of flukes is not identical to that of any of the monospecific anti-fluke antisera produced by Tailliez and Korach (1970b), Hillyer (1980) or Yoshihara *et al.* (1979). However, there is some support for the view that an antibody of the same specificity may be found in the sera of infected animals. All infected sera bind to the caeca and tegument (Thorpe 1965b; Movsesijan and Cuperlovic 1970; Borojevic *et al.* 1973; Rajasekariah and Howell 1978b; Hanna 1980a, b), as does MF1 antibody (Figs. 4.3 and 4.5), but Thorpe (1965b) and Yoshihara *et al.* (1979) found that, like MF1 antibody (Figs. 4.4 and 4.5), infected sera also bound to testes tubules.

Chapter 5

GENERAL DISCUSSION

The slow progress towards isolating functional antigens and antigens with immunodiagnostic potential is hindering the development of immunological aids for the control of parasitic diseases (Kagan 1979; Urquhart 1980; Lloyd 1981). However, hybridoma technology may hasten the advent of solutions to this problem. This new technology provides a powerful tool for resolving the antigenic complexity of parasites because it bypasses many of the restrictions of physicochemical and classical immunological methods. The present study applied hybridoma technology to the study of fascioliasis. Methods were developed for producing and screening hybridomas and analysing their products. The latter included attempts to use MCA to study individual fluke antigens.

Several methods, including indirect haemagglutination and haemolytic plaque assays, failed to detect anti-fluke MCA in hybrid cell culture supernatants. However, an RIA was developed which was a successful detection method. The fluorescent antibody technique described in Chapter 4 might also be useful for screening hybrid cells.

The hybridoma chosen for detailed study (MF1) secreted murine IgG₁ which bound in RIA to soluble antigens derived from several helminths including *S. mansoni*. In addition, using immunofluorescence MF1 antibody was found to bind to portions of the caeca, uterus, testes and tegument of adult flukes. Despite many attempts,

involving 3 different methods, its corresponding fluke antigen was not isolated. Nevertheless, the employment of MCA other than MF1 or modifications to the isolation methods may achieve this goal (see Section 4.4).

Although the approach used in the present study produced anti-*F. hepatica* MCA, different strategies could increase yields of hybridomas of value for the immunodiagnosis and immunoprophylaxis of fascioliasis. It was argued in Section 2.4 that artificial immunization, rather than infection, of spleen cell donors, may produce greater yields of hybrid cells having the desired specificity. Moreover, if an immunodiagnostic reagent for use with sheep sera were to be made, it may be advisable to purify (using a battery of antisera and affinity chromatography) the subset of fluke antigens that sheep respond to during infection for use as the immunogen. This procedure might avoid the problem that murine MCA produced using mice immunized with crude antigen preparations may be directed at determinants that sheep do not normally respond to during infection (Craig *et al.* 1980). There is evidence that this could be a problem in similar work with flukes, particularly if infected animals are used as spleen cell donors. Lehner and Sewell (1980) have shown that there are qualitative differences between the sets of fluke antigens that elicit humoral responses in infected sheep, rats and mice.

The ideal reagents for the immunodiagnosis of fascioliasis in sheep would be sheep MCA, but they are difficult to produce. The result of attempts to produce them in the present study was a sheep x

mouse hybridoma that secreted sheep Ig of unknown specificity.

Although this appears to be the first recorded source of a monoclonal sheep Ig (see Section 1.3.5), it lost chromosomes during prolonged culture. This is usually coincident with a loss of Ig secretion by interspecific hybrid cells, but this was not investigated in the present study.

It may be necessary to adopt other approaches to produce sheep MCA of predetermined specificity. One approach which might succeed is to adapt the methods of producing rabbit MCA developed by Businaro *et al.* (1981). This would involve both culturing the fusion products with sheep fibroblast cells and screening hybrid cells within a week of fusion to shorten the time in which cells could lose chromosomes before they are cloned. An alternative strategy might be to produce sheep myelomas, perhaps by transforming B cells *in vitro*, and selecting one that is suitable for fusion. Sheep x sheep hybridomas would be expected to be more stable than interspecific ones.

Testing MCA for species specificity requires a large panel of antigens from parasites which are both closely and distantly related phylogenetically, as well as sera - for inhibition assays - from animals infected with these parasites (Craig *et al.* 1980). The latter are particularly necessary if a monoclonal antibody appears to be species specific using the antigens of a range of parasites because concentrations of crossreacting antigens in a parasite may be below the level of detection but might nevertheless elicit strong humoral responses. Unfortunately, the species specificity of anti-parasite MCA has rarely been checked. Moreover, in the few cases that it has

been investigated the above-mentioned criteria have not been met. Indeed, some claims of species specificity have been based on tests using only one or two members of the same genus (Pearson *et al.* 1980, 1981; Perrin *et al.* 1980, 1981) or of other groups (Mitchell *et al.* 1979; Sethi *et al.* 1980). Furthermore, of the MCA found not to be species specific (Craig *et al.* 1980; Hillyer and Pelley 1980; Perrin *et al.* 1980, 1981), only those studied by Craig *et al.* (1980) were tested for crossreactivity outside the genus. Therefore, it is likely that many MCA have been produced which, like the MF1 antibody, are directed at antigens shared by a wide range of parasites. They should also be tested for their ability to bind to intermediate and definitive host antigens.

Species specificity testing of MCA is essential if immunodiagnostic reagents are being developed. In these cases, crossreactivity with those parasitic infections that can occur in the same host must be checked. It appears that only the studies by Craig *et al.* (1980) and Mitchell *et al.* (1981) have met this requirement.

When developing immunodiagnostic reagents it may be found that 2 or more MCA are necessary in order to cancel out the false negative tests that may be produced if subpopulations of infected individuals respond to different species-specific antigens. In addition, immunodiagnostic reagents might be developed by removing crossreactive antigens rather than purifying species-specific ones. However, the separation of crossreactive and specific determinants could be difficult if they are on the same molecule.

A successful strategy for developing a specific, sensitive immunodiagnostic test for fascioliasis may be to produce MCA to fraction II, a circulating antigen (see Section 1.2.5) which appears to be specific for fascioliasis (Robert *et al.* 1980). An RIA or ELISA of sera could be developed if these MCA were both able to bind to PVC and to act as the I^{125} - or enzyme-labelled tracer. This type of approach has been applied to the diagnosis of toxoplasmosis (Araujo *et al.* 1980). In the case of *F. hepatica*, Ambroise-Thomas *et al.* (1980) have shown that an ELISA, employing a rabbit antiserum to a crude fluke homogenate, was able to detect fluke antigens in the sera of 88.2% of cattle carrying natural, homologous infections. The existence of crossreactivity problems when measuring levels of circulating antigens produced by helminth infections has not yet been tested. Therefore, the need for MCA to replace antisera in order to overcome such problems, if they occur, is currently unknown.

As discussed in Section 1.2, rats, unlike mice and sheep, acquire immunity to *F. hepatica*. Therefore, attempts to produce MCA to functional fluke antigens should use a rat myeloma and rats injected with crude metacercarial, juvenile or adult antigens or the immunoprecipitated functional antigens studied by Howell (1979) and Howell and Sandeman (1979). It appears that all developmental stages of *F. hepatica* possess similar antigens (Sandeman and Howell 1981a; Irving and Howell 1982a), and would be suitable sources of immunogen. Following fusion, hybrid cell supernatants could be screened with a fluorescent antibody test or RIA (see Section 4.2). Initial screening for MCA to tegumental antigens, which appear to be targets of immune

attack (Bennett *et al.* 1980; Davies and Goose 1981), could employ solubilised tegument (Hillyer 1980) or incubation products, which contain tegumental antigens (Howell and Sandeman 1979; Sandeman and Howell 1980). The fluorescent antibody test may be more appropriate as a secondary screening assay because it is more time consuming than the RIA and probably not as sensitive. It would be advantageous to screen for the desired MCA as soon as possible after fusion because they could be expected to appear alongside a large excess of unsuitable anti-*F. hepatica* MCA.

Anti-fluke MCA could have numerous applications. MCA to functional antigens would facilitate dissection of the rat's immune responses to this parasite. MCA to immunosuppressive, parasite-derived molecules (which may be functional antigens), the occurrence of which has been suggested by the work of Goose (1978) and Sandeman and Howell (1980, 1981b), would also be valuable. In addition, the chemotherapy of fascioliasis might be more efficient if an anthelmintic or other poison were conjugated with MCA. The antibody should ensure that the drug is concentrated on or in the parasite. The ensuing drug-induced damage may render the parasite more susceptible to immune effectors.

After specific or functional fluke antigens have been isolated and identified, sources of large quantities of purified antigen will be required for various purposes, especially for vaccination. Since helminth parasites cannot be grown satisfactorily *in vitro*, alternative methods of producing large amounts of antigens need to be developed. Howell (1981) has discussed 3 approaches that might be

successful. These are the production of (1) fluke-derived cell lines; (2) hybrid cell lines formed by fusing fluke cells with malignant cells; (3) bacterial clones that synthesize fluke antigens, using recombinant DNA technology. MCA may be particularly useful in assays designed to determine which clones of fluke cells, hybrid cells or recombinant DNA-containing bacteria are secreting parasite antigens. In addition, all 3 approaches will probably require purification of the parasite antigens they produce. This may be most readily achieved by affinity chromatography using MCA.

BIBLIOGRAPHY

- Aalund O. and P. Nansen 1972 Migration inhibition testing on peripheral leukocytes from liver fluke infected rabbits. *Acta Vet. Scand.* 13: 591-593.
- Aalund O., K. Nielsen and L. Eriksen 1972 Migration inhibition studies on peripheral leukocytes and lymph node cells from ruminants with fascioliasis. *Acta Vet. Scand.* 13: 290-291.
- Abbas A.K., S.L. James and A. Sher 1981 Immunogenicity of haptenated schistosomula *in vitro*. *J. Immunol.* 126: 1022-1024.
- Abrahamson D.R., A. Powers and R. Rodewald 1979 Intestinal absorption of immune complexes by neonatal rats: A route of antigen transfer from mother to young. *Science* 206: 567-569.
- Albright J.W. and J.F. Albright 1981 Inhibition of murine humoral immune responses by substances derived from trypanosomes. *J. Immunol.* 126: 300-303.
- Alger N.E. and J. Harant 1976 *Plasmodium berghei*: Protection against sporozoites by normal mosquito tissue vaccination of mice. *Expl. Parasitol.* 40: 269-272.
- Allison A.C. and I.A. Clark 1978 Macrophage activation and its relevance to the immunology of parasitic diseases. *INSERM* 72: 147-160.
- Allison A.C., P. Davies and I.A. Clark 1977 Cellular interaction in fibrogenesis. *Ann. Rheum. Dis.* 36: 8-13.

- Altman A. and D. Katz 1980 Production and isolation of helper and supressor factors. *J. Immunol. Methods* 38: 9-41.
- Ambroise-Thomas P., P.T. Desgeorges and M. Bouttaz 1980 Le diagnostic immuno-enzymologique (ELISA) de la fasciolase humaine et bovine. Détection d'anticorps et/ou d'antigenes circulants. *Ann. Soc. Med. Trop.* 60: 47-60.
- Anderson J.C., D.L. Hughes and E. Harness 1975 The immune response of rats to subcutaneous implantation with *Fasciola hepatica*. *Br. Vet. J.* 131: 509-518.
- Andersson J. and F. Melchers 1978 The antibody repertoire of hybrid cell lines obtained by fusion of X63-Ag8 myeloma cells with mitogen-activated B cell blasts. *Curr. Top. Microbiol. Immunol.* 81: 130-139.
- Andres G.A. 1979 Immune complex diseases. In: N.R. Rose, F. Milgrom and C.J. Von Oss *Principles of Immunology*, 2nd Edition, MacMillan Publishing Co., New York, p. 319-337.
- Andrzejewski C., B.D. Stollar, T.M. Lalor and R.S. Schwartz 1980 Hybridoma autoantibodies to DNA. *J. Immunol.* 124: 1499-1502.
- Anwar A.R.E., S.R. Smithers and A.B. Kay 1979 Killing of schistosomula of *Schistosoma mansoni* coated with antibody and/or complement by human leucocytes *in vitro*: Requirement for complement in preferential killing by eosinophils. *J. Immunol.* 122: 628-637.
- Araujo F.G., E. Handman and J.S. Remington 1980 Use of monoclonal antibodies to detect antigens of *Toxoplasma gondii* in serum and other body fluids. *Infect. Immun.* 30: 12-16.

- Araujo F.G. and J.S. Remington 1980 Antigenemia in recently acquired acute toxoplasmosis. *J. Infec. Dis.* 141: 144-150.
- Armour J. and J.D. Dargie 1974 Immunity to *Fasciola hepatica* in the rat. Successful transfer of immunity by lymphoid cells and by serum. *Expl. Parasitol.* 35: 381-388.
- Askenase P.W. 1977 Role of basophils, mast cells and vasoamines in hypersensitivity reactions with a delayed time course. *Prog. Allergy* 23: 240-347.
- Astaldi G.C.B., M.C. Janssen, P. Landsdorp, C. Williams, W.P. Zeijlemaker and F. Oosterhof 1980 Human endothelial culture supernatant (HECS): A growth factor for hybridomas. *J. Immunol.* 125: 1411-1414.
- Auriault C., M. Joseph, J.P. Dessaint and A. Capron 1980 Inactivation of rat macrophages by peptides resulting from cleavage of IgG by schistosoma larvae proteases. *Immunol. Lett.* 2: 135-139.
- Auriault C., M.A. Ouaisi, G. Torpier, H. Eisen and A. Capron 1981 Proteolytic cleavage of IgG bound to the Fc receptor of *Schistosoma mansoni* schistosomula. *Parasite Immunol.* 3: 33-44.
- Avraham H., D.T. Spira, Y. Gorsky and D. Sulitzeanu 1980 A solid-phase antibody binding-inhibition test, for the assay of *Plasmodium berghei* antigen and antibodies, using radioiodinated protein A. *J. Immunol. Methods* 32: 151-155.
- Babenskas N., J. Pagirays and V. Allisauskaite 1958 Diagnosis of fascioliasis in farm animals and methods for its control in the Lithuanian S.S.R. *Acta Parasit. Lith.* 1: 121-133.

- Bankert R.B., D. Des Soye and L. Powers 1980 Screening and replica plating of anti-hapten hybridomas with a transfer template hemolytic spot assay. *J. Immunol. Methods* 35: 23-32.
- Barger I.A., K.M. Dash and W.H. Southcott 1978 Epidemiology and control of liverfluke in sheep. In: A.D. Donald, W.H. Southcott and J.K. Dineen *The Epidemiology and Control of Gastrointestinal Parasites of Sheep in Australia*. Division of Animal Health, CSIRO, Australia, p. 65-74.
- Barnstable C.J. 1980 Monoclonal antibodies which recognise different cell types in the rat retina. *Nature* 286: 231-235.
- Baron R.W. and C.E. Tanner 1977 *Echinococcus multilocularis* in the mouse: The *in vitro* protoscolicidal activity of peritoneal macrophages. *Int. J. Parasitol.* 7: 489-495.
- Bartlett A., D.E. Bidwell and A. Voller 1975 Preliminary studies on the application of enzyme-immunoassay in the detection of antibodies in onchocerciasis. *Tropenmed. Parasitol.* 26: 370-374.
- Bawden M.P. and T.H. Weller 1974 *Schistosoma mansoni* circulating antigen: Detection by complement fixation in sera from infected hamsters and mice. *Am. J. Trop. Med. Hyg.* 23: 1077-1084.
- Beachy J.C. and M.P. Czech 1980 Production of insulinomimetic antibodies against rat adipocyte membranes by hybridoma cells. *J. Supramol. Struct.* 13: 447-456.
- Bechtol K.B., S.C. Brown and R.H. Kennett 1979 Recognition of differentiation antigens of spermatogenesis in the mouse by using antibodies from spleen cell-myeloma hybrids after syngeneic immunisation. *Proc. Natl. Acad. Sci.* 76: 363-367.

- Behm C. and C. Bryant 1980 Regulatory properties of a partially purified preparation of pyruvate kinase from *Fasciola hepatica*. *Int. J. Parasitol.* 10: 107-114.
- Belozyorov S.N., A.S. Bessonov, A.I. Vishnyauskas and N.V. Shekhovtsov 1978 Vital diagnosis of trichinellosis using radioactive labelled antibodies. In: C.W. Kim and Z.S. Pawlowski *Trichinellosis*. University Press of New England, Hanover, New Hampshire, USA, p. 375-379.
- Benex J. 1964 Serological test for fascioliasis. Slide agglutination with antigen absorbed on latex particles. *Bull. Soc. Path. Exot.* 57: 495-502.
- Benex J., L. Lamy and J. Geldel 1959 Étude de la réaction de fixation du complément à l'antigène distomien chez le mouton. *Bull. Soc. Path. Exot.* 52: 83-87.
- Ben-Ismail R., B. Carme and M. Gentilini 1979 Mise en évidence de substance P1 ou P1-like dans *Fasciola hepatica* et *Fasciola gigantica*. *Path. Biol.* 27: 487-489.
- Ben-Ismail R., B. Carme, G. Neil and M. Gentilini 1980 Non-specific serological reactions with *Echinococcus granulosus* antigens: Role of anti-P1 antibodies. *Am. J. Trop. Med. Hyg.* 29: 239-245.
- Ben-Ismail R., P. Rouger, B. Carme, M. Gentilini and C. Salmon 1980 Comparative automated assay of anti-P antibodies in acute hepatic distomiasis (Fascioliasis) and in hydatidosis. *Vox Sang.* 38: 165-168.

- Bennett C.E. 1978 The identification of soluble adult antigen on the tegumental surface of juvenile *Fasciola hepatica*. *Parasitology* 77: 325-332.
- Bennett C.E. and L.T. Threadgold 1973 Electron microscope studies of *Fasciola hepatica*. XIII. Fine structure of newly excysted juvenile. *Expl. Parasitol.* 34: 85-89.
- Bennett C.E. and L.T. Threadgold 1975 *Fasciola hepatica*: Development of the tegument during migration in the mouse. *Expl. Parasitol.* 38: 38-55.
- Bennett C.E., D.L. Hughes and E. Harness 1980 *Fasciola hepatica*: Changes in tegument during killing of adult flukes surgically transferred to sensitized rats. *Parasite Immunol.* 2: 39-55.
- Berggren W.L. and T.H. Weller 1967 Immuno-electrophoretic demonstration of a specific circulating antigen in animals infected with *Schistosoma mansoni*. *Am.J. Trop. Med. Hyg.* 16: 606-612.
- Bernhard H.P. 1976 The control of gene expression in somatic cell hybrids. *Int. Rev. Cytol.* 47: 289-325.
- Biancifiori F., T. Frescura, L. Gialletti and A. Morozzi 1980 Solid-phase micro-radioimmunoassay for antibodies to *Trichinella spiralis*. *Arch. Vet. Ital.* 31: 44-47.
- Bickle Q.D., M.G. Taylor, M.J. Doenhoff and G.S. Nelson 1979 Immunization of mice with gamma-irradiated intramuscularly injected schistosomula of *Schistosoma mansoni*. *Parasitology* 79: 209-222.

- Biguet J., F. Rose and R. Havez 1960 Les possibilités d'application de l'immunoélectrophorèse à l'étude des fractions antigeniques des helminthes. *C.R. Acad. Sci. Paris* 251: 982-984.
- Biguet J.L., A. Capron and P. Tran Van Ky 1962a Les antigènes de *Fasciola hepatica*. Etude électrophorétique et immunoélectrophorétique. Identification des fractions et comparaison avec les antigènes correspondant à sept autres helminthes. *Ann. Parasitol. Hum. Comp.* 37: 221-231.
- Biguet, J., A. Capron, P. Tran Van Ky and R. D'Haussey 1962b Etude immunoélectrophorétique comparée des antigènes de diverse helminthes. *C.R. Acad. Sci. Paris* 254: 3.600-3.602.
- Biguet J.L., A. Capron, P. Tran Van Ky and F. Rose 1965a Présence de substances de type C dans les antigènes vermineux et de protéine anti-C au cours des helminthiases humaines ou expérimentales. I. Etude immunologique préliminaire et répercussions pratiques. *Rev. Immunol. Paris* 29: 233-240.
- Biguet J., G. Rose and A. Capron 1965b Le diagnostic de la distomatose à *Fasciola hepatica* par la réaction d'haémagglutination. Comparaison avec les résultats de l'immunoélectrophorèse et de la réaction d'hémolyse. *Bull. Soc. Path. Exot.* 58: 866-878.
- Biguet J., F. Rose, A. Capron and P. Tran Van Ky 1965c Contribution de l'analyse immunoélectrophorétique à la connaissance des antigènes vermineux. Incidences pratiques sur leur standardisation, leur purification et le diagnostic des helminthiases par immunoélectrophorèse. *Rev. Immunol. Paris* 29: 5-30.

- Blann A.D. 1979 Cell hybrids: An important new source of antibody production. *Med. Lab. Sci.* 36: 329-338.
- Bloom B.R. 1979 Games parasites play: How parasites evade immune surveillance. *Nature* 279: 21-26.
- Bolbol A-S., J.A. Hammond and M.M.H. Sewell 1978 The response of rabbits to repeated infections with *Fasciola hepatica*. *Vet. Sci. Comm.* 2: 231-235.
- Bolton A.E. 1977 Radioiodination Techniques. The Radiochemical Centre, Amersham, England.
- Bommeli W., V. Kihm, F. Zindel and H. Fey 1980 Enzyme linked immunoassay and fluorescent antibody techniques in the diagnosis of viral diseases using staphylococcal protein A instead of anti-gammaglobulins. *Vet. Immunol. Immunopathol.* 1: 179-193.
- Boray J.C. 1967a Studies on experimental infections with *Fasciola hepatica* with particular reference to acute fascioliasis in sheep. *Ann. Trop. Med. Parasitol.* 61: 439-450.
- Boray J.C. 1967b The effect of host reactions to experimental *Fasciola hepatica* infections in sheep and cattle. *Vet. Med. Rev.* 84-96.
- Boray J.C. 1969 Experimental fascioliasis in Australia. *Adv. Parasitol.* 7: 95-210.
- Boray J.C. 1978 The potential impact of exotic *Lymnaea* spp. on fascioliasis in Australasia. *Vet. Parasitol.* 4: 127-141.

- Boray J.C. and K. Enigk 1964 Laboratory studies on the survival and infectivity of *Fasciola hepatica* and *Fasciola gigantica* metacercariae. *Z. Tropenmed. Parasitol.* 15: 324-331.
- Bordenave G.R. 1979 Characteristics of the antigenic determinants revealed by hybridoma made monoclonal antibodies against the Fc fragment of rabbit IgG. *Immunol. Lett.* 1: 165-171.
- Borojevic D., B. Jovanovic and M. Movsesijan 1973 Fluorescent antibody studies of *Fasciola hepatica* infections. *Acta Vet. Beograd* 23: 55-62.
- Bout D., J.C. Dugimont, H. Farag and A. Capron 1976 Immunodiagnosis of human parasitic diseases by ELISA. In: G. Feldman, P. Druet, J. Bignon and S. Avrameas *Immunoenzymatic Techniques*. North Holland, Amsterdam, p. 175-180.
- Bout D., H. Dupas, Y. Carlier, D. Afchain and A. Capron 1977a High resistance induced by young live bacillus Calmette-Guérin (BCG) to *Schistosoma mansoni* infection in mice. *Ann. Immunol. Paris* 128C: 811-816.
- Bout D., J-P. Dessaint, H. Dupas, L. Yarzabal and A. Capron 1977b Characterization of allergens in *Schistosoma mansoni*, *Fasciola hepatica* and *Echinococcus granulosus*. *Ann. Immunol. Paris* 128C: 687-698.
- Bout D.T., M. Joseph, J.R. David and A. Capron 1981 *In vitro* killing of *S. mansoni* schistosomula by lymphokine-activated mouse macrophages. *J. Immunol.* 127: 1-5.

Brodsky F.M., P. Parham, C.J. Barnstable, M.J. Crumpton and W.F.

Bodmer 1979 Monoclonal antibodies for analysis of the HLA system. *Immunol. Rev.* 47: 3-62.

Brooks C.G., P.J. Webb, R.A. Robins, G. Robinson, R.W. Baldwin and

M.F.W. Festing 1980 Studies on the immunobiology of rnu/rnu "nude" rats with congenital aplasia of the thymus. *Eur. J. Immunol.* 10: 58-65.

Brooks D.A., I. Beckman, J. Bradley, P.J. McNamara, M.E. Thomas and

H. Zola 1980 Human lymphocyte markers defined by antibodies derived from somatic cell hybrids. I. A hybridoma secreting antibody against a marker specific for human B lymphocytes. *Clin. Exp. Immunol.* 39: 477-485.

Brower D.L., R.J. Smith and M. Wilcox 1980 A monoclonal antibody specific for diploid epithelial cells in *Drosophila*. *Nature* 285: 403-405.

Brown J.P., J.D. Tamerius and I. Hellström 1979 Indirect ^{125}I -labelled protein A assay for monoclonal antibodies to cell surface antigens. *J. Immunol. Methods* 31: 201-209.

Bryz-Gornia C., C. Long, T. Daly, M. Klinger and W. Weidanz 1981 Antibody reactivities arising during experimental malaria. *Fed. Proc.* 40: 1073.

Bundesen P.G. and P.A. Janssens 1971 Biochemical tracing of parasitic infections. I. *Fasciola hepatica* L. in mice - a qualitative study. *Int. J. Parasitol.* 1: 7-14.

Burden D.J. and N.C. Hammet 1978 Microplate enzyme linked immunosorbent assay for antibody to *Fasciola hepatica* in cattle. *Vet. Rec.* 103: 158.

- Burden D.J. and N.C. Hammet 1980 *Fasciola hepatica*: Attempts to immunize rats using fluke eggs and *in vitro* culture products. *Vet. Parasitol.* 7: 51-57.
- Burden D.J., D.L. Hughes, N.C. Hammet and K.A. Collis 1978 Concurrent daily infection of calves with *Fasciola hepatica* and *Ostertagia ostertagi*. *Res. Vet. Sci.* 25: 302-306.
- Burnet F.M. 1959 The Clonal Selection Theory of Acquired Immunity. Cambridge University Press, Cambridge.
- Businaro R., R.H. Butler, A.E. Rubenstein and R.P. Revoltella 1981 Monoclonal antibodies against mouse nerve growth factor produced by somatic cell hybrids. *J. Neurosci. Res.* 6: 89-98.
- Butterworth A.E., R.F. Sturrock, V. Houba., A.A.F. Mahmoud, A. Sher and P.H. Rees 1975 Eosinophils as mediators of antibody-dependent damage to Schistosomula. *Nature* 256: 727-729.
- Butterworth A.E., J.R. David, D. Franks, A.A.F. Mahmoud, P.H. David, R.F. Sturrock and V. Houba 1977 Antibody-dependent eosinophil-mediated damage to ^{51}Cr -labelled schistosomula of *Schistosoma mansoni*. *J. Exp. Med.* 145: 136-150.
- Butterworth A.E., M.A. Vadas, E. Martz and A. Sher 1979a Cytolytic T lymphocytes recognise alloantigens on schistosomula of *Schistosoma mansoni*, but fail to induce damage. *J. Immunol.* 122: 1314-1321.
- Butterworth A.E., D.L. Wassom, G.J. Gleich, D.A. Loegering and J.R. David 1979b Damage to schistosomula of *Schistosoma mansoni* induced directly by eosinophil major basic protein. *J. Immunol.* 122: 221-229.

Campbell N.J., J.D. Kelly, R.B. Townsend and J.K. Dineen 1977

The stimulation of resistance in sheep to *Fasciola hepatica* by infection with *Cysticercus tenuicollis*. *Int. J. Parasitol.* 7: 347-351.

Campbell N.J., P. Gregg, J.D. Kelly and J.K. Dineen 1978a

Failure to induce homologous immunity to *Fasciola hepatica* in sheep vaccinated with irradiated metacercariae. *Vet. Parasitol.* 4: 143-152.

Campbell N.J., J.D. Kelly and J.K. Dineen 1978b The effect of

age of host in resistance expressed at the gut level in rats infected with *Fasciola hepatica*. *Vet. Parasitol.* 4: 317-325.

Campbell N.J., J.K. Dineen and J.D. Kelly 1979 The effect of

Taenia hydatigena infection on existing and concurrent infections of *Fasciola hepatica* in sheep. *Res. Vet. Sci.* 26: 391-393.

Campbell W.C. and T.A. Barry 1970 A biochemical method for the

detection of anthelmintic activity against liverfluke (*Fasciola hepatica*). *J. Parasitol.* 56: 325-331.

Capron A. and M.A. Lesoni 1969 Rôle protecteur du BCG dans la

schistosomiase expérimentale à *S. mansoni*. *C.R. Hebd. Seanc. Acad. Sci. Paris Ser. D* 269: 2110-2112.

Capron A., J. Biguet, P. Tran Van Ky and G. Rose 1964 Possibilités

nouvelles dans le diagnostic immunologique de la distomatose humaine à *Fasciola hepatica*. Mise en évidence d'anticorps sériques par immunoélectrophorèse. *Presse Med.* 72: 3103-3107.

- Capron A., G. Rose, G. Luffau, J. Biguet and F. Rose 1965
 Apport de la distomatose expérimentale à la connaissance de
 la distomatose humaine à *Fasciola hepatica*. Aspects
 immunologiques. *Rev. Immunol. Paris* 29: 25-41.
- Capron A., J. Biguet, A. Vernes and D. Afchain 1968 Structure
 antigénique des helminthes. Aspects immunologiques des relations
 hôte-parasite. *Path. Biol. Paris* 16: 121-138.
- Capron A., A. Vernes and J. Fruit 1971 Le diagnostic immunologique
 de la distomatose hépatique à *F. hepatica*. Bilan personnel à
 propos de 430 observations. Etude comparée de trois techniques
 diagnostiques. *Rev. Pathol. Comp.* 70: 31-36.
- Capron A., M. Capron, H. Dupas, D. Bout and A. Petiprez 1974 Etude
in vitro des phenomenes immunologiques dans la schistosomiase
 humaine et expérimentale. I. Etude comparative *in vitro*
 l'activite lethale adultes de *Schistosoma mansoni*. *Int. J.*
Parasitol. 4: 613-623.
- Capron A., J.C. Dugimont, J. Fruit and D. Bout 1975a Application of
 immunoenzyme methods in diagnosis of human parasitic diseases.
Ann. N.Y. Acad. Sci. 254: 331.
- Capron A., J-P. Dessaint, M. Capron and H. Bazin 1975b Specific
 IgE antibodies in immune adherence of normal macrophages to
Schistosoma mansoni schistosomules. *Nature* 253: 474-475.
- Capron A., J-P. Dessaint, M. Joseph, R. Rousseaux, M. Capron and
 H. Bazin 1977 Interaction between IgE complexes and macrophages
 in the rat: A new mechanism of macrophage activation. *Eur. J.*
Immunol. 7: 315-322.

- Capron A., J-P. Dessaint and M. Capron 1978 Effector mechanisms in immunity to schistosomes. *INSERM* 72: 217-230.
- Capron A., J-P. Dessaint and M. Capron 1980 Immunoregulation des infections par schistosomes. *Med. Trop.* 40: 243-249.
- Capron M., A. Capron, G. Torpier, H. Bazin, D. Bout and M. Joseph 1978a Eosinophil-dependent cytotoxicity in rat schistosomiasis. Involvement of IgG_{2a} antibody and role of mast cells. *Eur. J. Immunol.* 8: 127-133.
- Capron M., J. Rousseaux, C. Mazinque, H. Bazin and A. Capron 1978b Rat mast cell-eosinophil interaction in antibody-dependent eosinophil cytotoxicity to *Schistosoma mansoni* schistosomula. *J. Immunol.* 121: 2518-2525.
- Capron M., G. Torpier and A. Capron 1979 *In vitro* killing of *Schistosoma mansoni* schistosomula by eosinophils from infected rats: Role of cytophilic antibodies. *J. Immunol.* 123: 2220-2230.
- Capron M., Y. Carlier, H. Nzeyimana, P. Minoprio, F. Santoro, B. Sellin and A. Capron 1980 *In vitro* study of immunological events in human and experimental schistosomiasis: Relationships between cytotoxic antibodies and circulating schistosoma antigens. *Parasite Immunol.* 2: 223-235.
- Carlier Y., D. Bout, J.C. Bina, D. Camus, J.F.M. Figueiredo and A. Capron 1975 Immunological studies in human schistosomiasis. I. Parasitic antigen in urine. *Am. J. Trop. Med. Hyg.* 24: 949-954.

- Carlier Y., D. Bout and A. Capron 1978 Further studies on the circulating M antigen in human and experimental *Schistosoma mansoni* infections. *Ann. Immunol. Paris* 129C: 811-818.
- Carlier Y., D. Bout and A. Capron 1979 Application de l'ELISA (Enzyme Linked Immunosorbent Assay) en parasitologie et mycologie. *Rev. Inst. Past. Lyon* 12: 25-33.
- Carlier Y., H. Nzeyimana, D. Bout and A. Capron 1980 Evaluation of circulating antigens by a sandwich radioimmunoassay, and of antibodies and immune complexes, in *Schistosoma mansoni*-infected African parturients and their newborn children. *Am. J. Trop. Med. Hyg.* 29: 74-81.
- Catt K. and G.W. Tregear 1967 Solid-phase radioimmunoassay in antibody-coated tubes. *Science* 158: 1570-1572.
- Chapman C.B. and G.F. Mitchell 1981a *Fasciola hepatica*: Comparative studies on fascioliasis in rats and mice. *Expl. Parasitol.* Submitted for publication.
- Chapman C.B. and G.F. Mitchell 1981b *Fasciola hepatica*: Proteolytic cleavage of immunoglobulins. *Int. J. Parasitol.* Submitted for publication.
- Chappell L.H. 1976 The nutrition of *Schistosoma* and *Fasciola*. *Parasitology* 73: xxii.
- Choi W.Y. and O.R. Lee 1979a Immuno-electrophoresis of *Fasciola hepatica*. *Kor. J. Parasitol.* 17: 73-80.
- Choi W.Y. and O.R. Lee 1979b Immuno-electrophoretic studies of several helminths. *Kor. J. Parasitol.* 17: 147-153.

- Christensen N.O., P. Nansen, F. Frandsen, A. Bjorneboe and J. Monrad 1978 *Schistosoma mansoni* and *Fasciola hepatica*: Cross-resistance in mice. *Expl. Parasitol.* 46: 113-120.
- Christensen N.O., J. Monrad, P. Nansen and F. Frandsen 1980 *Schistosoma mansoni* and *Fasciola hepatica*: Cross-resistance in mice with single-sex schistosome infections. *Expl. Parasitol.* 49: 116-121.
- Christensen P., A. Johansson and V. Nielson 1978 Quantitation of protein adsorbance to glass and plastics: Investigation of a new tube with low adherence. *J. Immunol. Methods* 23: 23-28.
- Civil R.H., K.S. Warren and A.A.F. Mahmoud 1978 Conditions for bacille Calmette-Guérin-induced resistance to infection with *Schistosoma mansoni* in mice. *J. Infec. Dis.* 137: 550-555.
- Clark W.A., A.W. Everett, F.W. Fitch, K.S. Frogner, S. Jakovcic, M. Rabinowitz, A.M. Warner and R. Zak 1980 Characterization of monoclonal antibodies directed against determinants on cardiac myosin heavy chain. *Bioc. Biop. Res. Comm.* 95: 1680-1686.
- Clegg J.A. and S.R. Smithers 1972 The effects of immune rhesus monkey serum on schistosomula of *Schistosoma mansoni* during cultivation *in vitro*. *Int. J. Parasitol.* 2: 79-98.
- Cleveland P.H., D.D. Richman, M.N. Oxman, P.S. Binder and D.M. Worthen 1979 Immobilization of viral antigens on filter paper for a ^{125}I Staphylococcal protein A immunoassay: A rapid and sensitive technique for detection of herpes simplex virus antigens and antiviral antibodies. *J. Immunol. Methods* 29: 369-386.

- Clevinger B., D. Hansburg and J. Davie 1978 Murine anti- α (1 \rightarrow 3) dextran antibody production by hybrid cells. *Curr. Top. Microbiol. Immunol.* 81: 110-114.
- Cocito C.G., B. Michot, J. Radovich and D.W. Talmage 1979 Immunosuppression by a mouse tumour resembles antigenic competition. *Proc. Natl. Acad. Sci. U.S.A.* 76: 2895-2897.
- Coffino P. and M.D. Scharff 1971 Rate of somatic mutation in immunoglobulin production by mouse myeloma cells. *Proc. Natl. Acad. Sci.* 68: 219-223.
- Coffman R.L. and I.L. Wiessman 1981 B220: A B cell-specific member of the T200 glycoprotein family. *Nature* 289: 681-683.
- Converse V.N. 1968 Investigations on the relationship of *Fasciola hepatica* miracidia to its molluscan host. *Wiad. Parazyt.* 14: 527.
- Cook W.D. and M.D. Scharff 1977 Antigen-binding mutants of mouse myeloma cells. *Proc. Natl. Acad. Sci.* 74: 5687-5691.
- Corba J. and R. Spaldonova 1975 Development of host resistance to *Fasciola hepatica* after the elimination of primary infection with Diamphenethide. *Folia Parasitol. (Praha)* 22: 133-139.
- Corba J., J. Armour, R.J. Roberts and G.M. Urquhart 1971 Transfer of immunity to *Fasciola hepatica* infection by lymphoid cells. *Res. Vet. Sci.* 12: 292-295.
- Cornish R.A. and C. Bryant 1976 The metabolic integrity of *Fasciola hepatica* during *in vitro* maintenance. *Int. J. Parasitol.* 6: 387-392.
- Cotton R.G.H., D.S. Secher and C. Milstein 1973 Somatic mutation and the origin of antibody diversity. Clonal variability of the immunoglobulin produced by MOPC21 cells in culture. *Eur. J. Immunol.* 3: 135-140.

- Cottrell B.J., D. Humber and R.F. Sturrock 1980a An immuno-suppressive factor in the serum of patients with schistosomiasis. *Trans. R. Soc. Trop. Med. Hyg.* 74: 415-416.
- Cottrell B.J., R.F. Sturrock and M. Vanhoegaerden 1980b An immunosuppressive factor in the serum of baboons (*Papio anubis*) infected with *Schistosoma mansoni*. *Immunology* 39: 589-598.
- Coudert J., J.P. Garin, P. Ambroise-Thomas, T. Kien Truong, J. Despeignes and M.A. Poithier 1967 La réaction d'immuno-fluorescence sur coupes de *Fasciola hepatica*: Une nouvelle technique pour le séro-diagnostic de la distomatose. *Bull. Soc. Path. Exot.* 60: 71-79.
- Cowan N.J., D.S. Secher and C. Milstein 1974 Intracellular immunoglobulin chain synthesis in non-secreting variants of a mouse myeloma: Detection of inactive light-chain messenger RNA. *J. Mol. Biol.* 90: 691-701.
- Cox F.E.G. 1979 Death of a schistosome. *Nature* 278: 401-402.
- Cox F.E.G. 1980 Monoclonal antibodies and immunity to malaria. *Nature* 284: 304-305.
- Craig P.S. and M.D. Rickard 1980 Evaluation of 'crude' antigen prepared from *Taenia saginata* for the serological diagnosis of *T. saginata* cysticercosis in cattle using the enzyme-linked immunosorbent assay (ELISA). *Z. Parasitkd.* 61: 287-297.
- Craig P.S., G.F. Mitchell, K.M. Cruise and M.D. Rickard 1980 Hybridoma antibody immunoassays for the detection of parasitic infection: Attempts to produce an immunodiagnostic reagent for a larval taeniid cestode infection. *Aust. J. Exp. Biol. Med. Sci.* 58: 339-350.

- Craig P.S., R. Hocking, G.F. Mitchell and M.D. Rickard 1981
Murine hybridoma-derived antibodies in the processing of antigens for the immunodiagnosis of hydatid (*Echinococcus granulosus*) infection in sheep. *Parasitology*. In press.
- Croce C.M., B. Bakay, W.L. Nyhan and H. Koprowski 1973 Re-expression of the rat hypoxanthine phosphoribosyl-transferase gene in rat-human hybrids. *Proc. Natl. Acad. Sci.* 70: 2590-2594.
- Croce C.M., M. Shander, J. Martinis, L. Circurel, G.G. D'Ancona and H. Koprowski 1980a Preferential retention of human chromosome 14 in mouse x human B cell hybrids. *Eur. J. Immunol.* 10: 486-488.
- Croce C.M., A. Linnenbach, W. Hall, Z. Steplewski and H. Koprowski 1980b Production of human hybridomas secreting antibodies to measles virus. *Nature* 288: 488-489.
- Cuello A.C., G. Galfre and C. Milstein 1979 Detection of substance P in the central nervous system by a monoclonal antibody. *Proc. Natl. Acad. Sci.* 76: 3532-3536.
- Cuello A.C., G. Galfre and C. Milstein 1980 Development of a monoclonal antibody against a neuroactive peptide: Immunocytochemical applications. *Adv. Bioc. Psychopharm.* 21: 349-364.
- Cuperlovic K. 1972a Studies on the immune response to infection with the liver fluke *Fasciola hepatica*. *J. Parasitol.* 58: 1209-1210.
- Cuperlovic K. 1972b Metabolic antigen of *Fasciola hepatica* in immunodiagnosis of fascioliasis. *Acta Vet. Beograd.* 22: 219-222.

- Cuperlovic K. and R. Lalic 1970 Immunološka reakcija kunića na reinfekciju sa *Fasciola hepatica*. *Acta Vet. Belgrade* 20: 333-339.
- Cuperlovic K. and R. Lalic 1972 Studies of metabolic antigen activity of adult *Fasciola hepatica* by complement fixation test. *Acta Vet. Beograd*. 22: 195-199.
- Cuperlovic K., A. Sokolic and M. Movsesijan 1968 Uporedna procena 'punog' i 'preciscenog' antigena *Fasciola hepatica*. *Acta Vet. Beograd* 18: 357-362.
- Cuperlovic K., M. Movsesijan, D. Borojevic and R. Lalic 1970 Reakcija makroorganizma na višekratnu infekciju sa metacerkarijama *Fasciola hepatica*. *Acta Vet. Beograd* 20: 81-85.
- Cuperlovic K., K.I. Altaif and J.D. Dargie 1978 Genetic resistance to helminths: A possible relationship between haemoglobin type and the immune responses of sheep to non-parasitic antigens. *Res. Vet. Sci.* 25: 125-126.
- Damian R.T. 1964 Molecular mimicry: Antigen sharing by parasite and host and its consequences. *Am. Nat.* 98: 129-149.
- Damian R.T., N.D. Greene and W.J. Hubbard 1973 Occurrence of mouse alpha 2-macroglobulin-like antigenic determinants on *Schistosoma mansoni* adults, with evidence on their nature. *J. Parasitol.* 59: 64-73.
- Damiani G., M. Frascio, U. Benatti, A. Morelli, E. Zocchi, M. Fabbi, A. Bargellesi, S. Pontremoli and A. De Flora 1980 Monoclonal antibodies to human erythrocyte glucose 6-phosphate dehydrogenase. *Febs Lett.* 119: 169-173.

- Dargie J.D., J. Armour and G.M. Urquhart 1973 Studies on immunity to *Fasciola hepatica*. *Parasitology* 67: xxv.
- Dargie J.D., J. Armour, B. Rushton and M. Murray 1974 Immune mechanisms and hepatic fibrosis in fascioliasis. In: E.J.L. Soulsby *Parasitic Zoonoses*. Academic Press, New York, p. 249-271.
- Dargie J.D., C.I. Berry, P.H. Holmes, J.F.S. Reid, R. Breeze, M.G. Taylor, E.R. James and G.S. Nelson 1977 Immunization of sheep against a virulent strain of *Schistosoma mattheei* using a strain of *S. mattheei* attenuated by hamster passage. *J. Helminthol.* 51: 347-357.
- David J.R. and A.E. Butterworth 1979 Immunity to schistosomes. Advances and prospects. *Ann. Internal Med.* 91: 641-643.
- Davidson R.L. 1974 Control of expression of differentiated functions in somatic cell hybrids. In: R.L. Davidson and F. De la Cruz *Somatic Cell Hybridisation*, Raven, N.Y., p. 131-150.
- Davies C. 1978 The ultrastructure of the tegument and the digestive caeca of *in vitro* cultured metacercariae of *Fasciola hepatica*. *Int. J. Parasitol.* 8: 197-206.
- Davies C. and J. Goose 1981 Killing of newly excysted juveniles of *Fasciola hepatica* in sensitized rats. *Parasite Immunol.* 3: 81-96.
- Davies C., M.D. Rickard, Smyth J.D. and D.L. Hughes 1979 Attempts to immunize rats against infection with *Fasciola hepatica* using *in vitro* culture antigens from newly excysted metacercariae. *Res. Vet. Sci.* 26: 259-260.

- Dawes B. 1963 Some observations of *Fasciola hepatica* L. during feeding operations in the hepatic parenchyma of the mouse, with notes on the nature of liver damage in this host. *Parasitology* 53: 135-143.
- Dawes B. and D.L. Hughes 1964 Fascioliasis: The invasive stages of *Fasciola hepatica* in mammalian hosts. *Adv. Parasitol.* 2: 97-168.
- Dawes B. and D.L. Hughes 1970 Fascioliasis: The invasive stages in mammals. *Adv. Parasitol.* 8: 259-273.
- Day K.P., R.J. Howard, S.J. Prowse, C.B. Chapman and G.F. Mitchell 1979 Studies on chronic versus transient intestinal nematode infections in mice I. A comparison of responses to excretory/secretory (ES) products of *Nippostrongylus brasiliensis* and *Nematospiroides dubius* worms. *Parasite Immunol.* 1: 217-239.
- De Heer D.H., J.M. Pages and A.E. Bussard 1980 Specificity of antierythrocyte autoantibodies secreted by a NZB-derived hybridoma and NZB peritoneal cells. *Cell. Immunol.* 49: 135-141.
- De Micco P., G. Pautrat, L. Benkoel, M.M. Pouget and J. Tamalet 1978 Kinetic and ultrastructural study of polyethylene glycol induced fusion of sheep fibroblasts - comparison with endogenous fusion induced by *Vesna* virus. *C.R. Soc. Biol.* 172: 122-129.
- Dean D.A. 1974 *Schistosoma mansoni*: Adsorption of human blood group A and B antigens by schistosomula. *J. Parasitol.* 60: 260-263.

- Dean D.A. 1977 Decreased binding of cytotoxic antibody by developing *Schistosoma mansoni*. Evidence for a surface change independent of host antigen adsorption and membrane turnover. *J. Parasitol.* 63: 418-426.
- Dean D.A. and K.W. Sell 1972 Surface antigens on *Schistosoma mansoni*. II. Adsorption of a Forssman-like host antigen by schistosomula. *Clin. Exp. Immunol.* 12: 525-540.
- Dean D.A., R. Wistar and K.D. Murrell 1974 Combined *in vitro* effects of rat antibody and neutrophilic leukocytes on schistosomula of *Schistosoma mansoni*. *Am. J. Trop. Med. Hyg.* 23: 420-428.
- Deelder A.M. 1973 Immunology of experimental infections with *Schistosoma mansoni* in the swiss mouse and with *Fasciola hepatica* in the rabbit. *Acta Leiden.* 39: 1-107.
- Deelder A.M., J.J. Snoijink and J.S. Ploem 1975 Experimental optimization of the DASS system for immunodiagnosis of some helminth infections. *Ann. N.Y. Acad. Sci.* 254: 119-134.
- Deelder A.M., H.T.M. Klappe, G.J.M.J. Van den Aardweg and E.H.E.M. Van Meerbeke 1976 *Schistosoma mansoni*: Demonstration of two circulating antigens in infected hamsters. *Expl. Parasitol.* 40: 189-197.
- Deelder A.M., E.J. Ruitenbergh, D. Kornelis and P.A. Steerenberg 1977 *Schistosoma mansoni*: Comparison of the immunoperoxidase techniques, DASS and ELISA, for human diagnosis. *Expl. Parasitol.* 41: 133-140.

- Deelder A.M., D. Kornelis, E.A.E. Van Marck, P.C. Eveleigh and J.G. Van Egmond 1980 *Schistosoma mansoni*: Characterisation of two circulating polysaccharide antigens and the immunological response to these antigens in mouse, hamster, and human infections. *Expl. Parasitol.* 50: 16-32.
- Delacroix D. and J.P. Vaerman 1979 Single purification of goat IgG₁ and IgG₂ subclasses by chromatography on protein A-sepharose at various pH. *Mol. Immunol.* 16: 837-840.
- Dennert G. 1971 The mechanism of antibody-induced stimulation and inhibition of the immune response. *J. Immunol.* 106: 951-956.
- Dessaint J.P., D. Camus, E. Fischer and A. Capron 1977 Inhibition of lymphocyte proliferation by factor(s) produced by *Schistosoma mansoni*. *Eur. J. Immunol.* 7: 624-629.
- Dessein A., J.C. Samuelson, A.E. Butterworth, M. Hogan, B.A. Sherry, M.A. Vadas and J.R. David 1981 Immune evasion by *Schistosoma mansoni*: Loss of susceptibility to antibody or complement-dependent eosinophil attack by schistosomula cultured in medium free of macromolecules. *Parasitology* 82: 357-374.
- Di Pauli R. and W.C. Raschke 1978 A hybridoma secreting IgM anti-IgI^b allotype. *Curr. Top. Microbiol. Immunol.* 81: 37-39.
- Diener E. and M. Feldman 1972 Relationship between antigen and antibody induced suppression of immunity. *Transplant. Rev.* 8: 76-103.
- Dimitrov G., K. K'ncheva and B. Georgiev 1978 The effect of the protein content in the diet on the development and pathogenicity of *Fasciola hepatica*. *Vet. Med. Nauki* 15: 92-100.

- Dineen J.K. and R.G. Windon 1980 The effect of sire selection on the responses of lambs to vaccination with irradiated *Trichostrongylus colubrifformis* larvae. *Int. J. Parasitol.* 10: 189-196.
- Dineen J.K., P. Gregg and A.K. Lascelles 1978 The response of lambs to vaccination with irradiated *Trichostrongylus colubrifformis* larvae: Segregation into 'responders' and 'non-responders'. *Int. J. Parasitol.* 8: 59-63.
- Dineen J.K., J.D. Kelly and N.J. Campbell 1978 Further observations on the nature and characteristics of cross protection against *Fasciola hepatica* produced in sheep by infection with *Cysticercus tenuicollis*. *Int. J. Parasitol.* 8: 173-176.
- Djeu J.Y., K.Y. Huang and R.B. Herberman 1980 Augmentation of mouse natural killer cell activity and induction of interferon by tumour cells *in vivo*. *J. Exp. Med.* 151: 781-789.
- Dodd K. 1969 Antigenic ontogeny of *Fasciola hepatica*. *Vet. Rec.* 85: 159.
- Dodd K. and T. O'Nuallain 1969 Effect of antilymphocytic sera on the histopathology of *Fasciola hepatica* infestation in rabbits. *J. Pathol.* 99: 335-337.
- Dorval G., K.I. Welsh and H. Wigzell 1975 A radioimmunoassay of cellular surface antigens of living cells using iodinated soluble protein A from *Staphylococcus aureus*. *J. Immunol. Methods* 7: 237-250.

- Doy T.G., D.L. Hughes and E. Harness 1978 Resistance of the rat to reinfection with *Fasciola hepatica* and the possible involvement of intestinal eosinophil leucocytes. *Res. Vet. Sci.* 25: 41-44.
- Doy T.G., D.L. Hughes and E. Harness 1980 The selective adherence of rat eosinophils to newly excysted *Fasciola hepatica* *in vitro*. *Res. Vet. Sci.* 29: 98-101.
- Doy T.G., D.L. Hughes and E. Harness 1981 The heterologous protection of rats against a challenge with *Fasciola hepatica* by prior infection with the nematode *Nippostrongylus brasiliensis*. *Parasite Immunol.* 3: 171-180.
- Doyle J.J. 1971 Acquired immunity to experimental infection with *Fasciola hepatica* in cattle. *Res. Vet. Sci.* 12: 527-534.
- Doyle J.J. 1972 Evidence of an acquired resistance in calves to a single experimental infection with *Fasciola hepatica*. *Res. Vet. Sci.* 13: 456-459.
- Doyle J.J. 1973a The relationship between duration of a primary infection and the subsequent development of an acquired resistance to experimental infections with *Fasciola hepatica* in calves. *Res. Vet. Sci.* 14: 97-103.
- Doyle J.J. 1973b Homocytotropic antibodies induced in calves by infection with *Fasciola hepatica*. *Internat. Arch. Allergy Appl. Immunol.* 45: 744-751.
- Doyle J.J. 1973c The precipitin response induced in calves by a single experimental infection with *Fasciola hepatica*. *Res. Vet. Sci.* 15: 119-120.

- Duffus W.P.H. and D. Franks 1980 *In vitro* effect of immune serum and bovine granulocytes on juvenile *Fasciola hepatica*. *Clin. Exp. Immunol.* 41: 430-440.
- Duffus W.P.H. and D. Franks 1981 The interaction *in vitro* between bovine immunoglobulin and juvenile *Fasciola hepatica*. *Parasitology* 82: 1-10.
- Duffus W.P.H., K. Thorne and R. Oliver 1980 Killing of juvenile *Fasciola hepatica* by purified bovine eosinophil proteins. *Clin. Exp. Immunol.* 40: 336-344.
- Duhamel R.C., E. Meezan and K. Brendel 1980 The pH-dependent binding of goat IgG₁ and IgG₂ to protein A sepharose. *J. Immunol. Methods* 31: 211-217.
- Durbin C.G. 1952 Longevity of the liver fluke, *Fasciola* sp. in sheep. *Proc. Helminthol. Soc. Wash.* 19: 120.
- Effros R.B., M.E. Frankel, W. Gerhard and P.C. Doherty 1979 Inhibition of influenza-immune T cell effector function by virus-specific hybridoma antibody. *J. Immunol.* 123: 1343-1346.
- Eilat D., R. Asofsky and R. Laskov 1980 A hybridoma from an autoimmune NZB/NZW mouse producing monoclonal antibody to ribosomal RNA. *J. Immunol.* 124: 766-768.
- Eisenbarth G.S., B.F. Haynes, J.A. Schroer and A.S. Fauci 1980 Production of monoclonal antibodies reacting with peripheral blood mononuclear cell surface differentiation antigens. *J. Immunol.* 124: 1237-1244.

- Eisenbarth G.S. 1981 Application of monoclonal antibody techniques to biochemical research. *Anal. Biochem.* 111: 1-16.
- Ellner J.J. and A.A.F. Mahmoud 1979 Killing of schistosomula of *Schistosoma mansoni* by normal human monocytes. *J. Immunol.* 123: 949-951.
- Engvall E. 1980 Enzyme immunoassay ELISA and EMIT. *Methods Enzymol.* 70: 419-439.
- Engvall E. and P. Perlmann 1972 Enzyme linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme labelled anti-immunoglobulin in antigen coated tubes. *J. Immunol.* 109: 129-135.
- Engvall E. and A.J. Pesce 1978 Quantitative enzyme immunoassay. *Scand. J. Immunol.* 8, Suppl. 7.
- Engvall E. and E. Ruoslahti 1979 Principles of ELISA and recent applications to the study of molecular interactions. In: R.M. Nakamura, W.R. Dito and E.S. Tucker III. *Immunoassays in the Clinical Laboratory*. Alan R. Liss Inc., N.Y., p. 89-97.
- Epstein N., L.H. Miller, D.C. Kaushel, I.J. Udeinya, J. Renner, R.J. Howard, R. Asofsky, M. Aikawa and R.L. Hess 1981 Monoclonal antibodies against a specific surface determinant on malarial (*Plasmodium knowlesi*) merozoites block erythrocyte invasion. *J. Immunol.* 127: 212-217.
- Erasmus D.A. 1977 The host-parasite interface of trematodes. *Adv. Parasitol.* 15: 201-242.

- Eriksen L. 1980a *Fasciola hepatica*: Influence of thymus function on the course of infections in mice. *Nord. Vet. Med.* 32: 243-254.
- Eriksen L. 1980b *Fasciola hepatica*: Influence of thymus function on the liver pathology in mice. *Nord. Vet. Med.* 32: 393-401.
- Eriksen L. and T. Flagstad 1974 *Fasciola hepatica*: Influence of extrahepatic adult flukes on infections and immunity in rats. *Expl. Parasitol.* 35: 411-417.
- Ershov V.S. 1959 The problem of immunization of domestic animals to helminthoses. *Congr. Mund. Vet.* 46: 279-289.
- Ershov V.S. and P.F. Poluektova 1978 Reagin-like antibodies in rabbits infected with *Fasciola hepatica*. *Trudy Vses. Instit. Gel'minthol. Skryabina* 24: 47-52.
- Eugui E.M. and A.C. Allison 1980 Differences in susceptibility of various mouse strains to haemoprotozoan infections: Possible correlation with natural killer activity. *Parasite Immunol.* 2: 277-292.
- Ey P.L., S.J. Prowse and C.R. Jenkin 1978 Isolation of pure IgG₁, IgG_{2a} and IgG_{2b} immunoglobulins from mouse serum using protein A-sepharose. *Immunochemistry* 15: 429-436.
- Fazekas de St Groth and D. Scheidegger 1980 Production of monoclonal antibodies: Strategy and tactics. *J. Immunol. Methods* 35: 1-21.

Ferreira A.W., A.L.M. Caldini, S. Hoshino-Shimizu and M.E.

Camargo 1979 Immuno-enzymatic assay for the detection of *Schistosoma mansoni* antigens in serum of mice harbouring bisexual or unisexual light worm infections. *J. Helminthol.* 53: 189-194.

Flagstad T. and L. Eriksen 1974 Hepatic immunoglobulin synthesis in *Fasciola hepatica* infected calves. *Res. Vet. Sci.* 17: 59-63.

Flagstad T., S. Anderson and K. Nielsen 1972 The course of experimental *Fasciola hepatica* infection in calves with a deficient cellular immunity. *Res. Vet. Sci.* 13: 468-475.

Fortmeyer H.P. 1973 Immunological studies on *Fasciola hepatica* infections of rabbits and on its localization. *Deut. Tier. Woch.* 80: 517-540.

Fraser C. and J.C. Venter 1980 Monoclonal antibodies to beta andrenergic receptors: Use in purification and molecular characterisation of beta receptors. *Proc. Natl. Acad. Sci.* 77: 7034-7038.

Freeman R.R., A.J. Trejdosiewicz and G.A.M. Cross 1980 Protective monoclonal antibodies recognising stage-specific merozoite antigens of a rodent malaria parasite. *Nature* 284: 366-368.

Gajos E. 1969 Etude des anticorps précipitants anti-*Fasciola hepatica* chez les bovins infestés et chez les lapins immunisés. *Arch. Inst. Past. Tunis* 46: 199-203.

Galfre G., S.C. Howe, C. Milstein, G.W. Butcher and J.C. Howard 1977 Antibodies to major histocompatibility antigens produced by hybrid cell lines. *Nature* 266: 550-552.

- Galfre G., C. Milstein and B. Wright 1979 Rat x rat hybrid myelomas and a monoclonal anti-Fd portion of mouse IgG. *Nature* 277: 131-133.
- Geftter M.L., D.H. Margulies and M.D. Scharff 1977 A simple method for polyethylene glycol-promoted hybridization of mouse myeloma cells. *Som. Cell Genet.* 3: 231-236.
- Gemmell M.A. and P.D. Johnstone 1981 Factors regulating tapeworm populations: Estimations of the duration of acquired immunity by sheep to *Taenia hydatigena*. *Res. Vet. Sci.* 30: 53-56.
- Gerhard W., T.J. Braciale and N.R. Klinman 1975 The analysis of the monoclonal immune response to influenza virus. I. Production of monoclonal anti-viral antibodies *in vitro*. *Eur. J. Immunol.* 5: 720-725.
- Gershon R.K., R.L. Carter and K. Kondo 1967 On concomitant immunity in tumour-bearing hamsters. *Nature* 197: 674-676.
- Gersten D.M. and J.J. Marchalonis 1978 A rapid, novel method for the solid-phase derivation of IgG antibodies for immune-affinity chromatography. *J. Immunol. Methods* 24: 305-309.
- Geyer E. 1967 Electrophoretische analysen an *Fasciola hepatica* - totalextrakten. *Z. Parasitkd.* 28: 352-369.
- Gisler R.H. 1978 Cellular regulation of immune responses. *INSERM* 72: 111-126.
- Glauert A.M. and Butterworth A.E. 1977 Morphological evidence for the ability of eosinophils to damage antibody-coated schistosomula. *Trans. R. Soc. Trop. Med. Hyg.* 71: 392-395.

- Glauert A.M., A.E. Butterworth, R.F. Sturrock and V. Houba 1978
The mechanism of antibody-dependent eosinophil-mediated damage to schistosomula of *Schistosoma mansoni* *in vitro*: A study by phase-contrast and electron microscopy. *J. Cell Sci.* 34: 173-192.
- Gleich G.J., G.M. Olson and H. Herlich 1979 The effect of antiserum to eosinophils on susceptibility and acquired immunity of the guinea-pig to *Trichostrongylus colubriformis*. *Immunology* 37: 873-880.
- Goding J.W. 1976a The chromic chloride method of coupling antigens to erythrocytes: Definition of some important parameters. *J. Immunol. Methods* 10: 61-66.
- Goding J.W. 1976b Conjugation of antibodies with fluorochromes: Modifications to the standard methods. *J. Immunol. Methods* 13: 215-226.
- Goding J.W. 1978 Use of staphylococcal protein A as an immunological reagent. *J. Immunol. Methods* 20: 241-253.
- Goding J.W. 1980 Antibody production by hybridomas. *J. Immunol. Methods* 39: 285-308.
- Goding J.W., V.T. Oi, P.P. Jones, L.A. Herzenberg and L.A. Herzenberg 1979 Monoclonal antibodies to alloantigens and to immunoglobulin allotypes. In: B. Pernis and H. Vogel *Cells of Immunoglobulin Synthesis*. Academic Press, N.Y., p. 309-331.
- Goetzl E.J. 1976 Modulation of human eosinophil polymorphonuclear leucocyte migration and function. *Am. J. Path.* 85: 419-435.

- Goetzl E.J. and K.F. Austen 1975 Purification and synthesis of eosinophilotactic tetrapeptides of human lung tissue: Identification as eosinophil chemotactic factor of anaphylaxis. *Proc. Natl. Acad. Sci.* 72: 4123-4127.
- Gold D. and J. Lengy 1972 Migratory behaviour and survival of transplanted adult *Fasciola hepatica* in the mouse peritoneal cavity. *J. Parasitol.* 58: 1009-1010.
- Gold R., F.S. Rosen and T.H. Weller 1969 A specific circulating antigen in hamsters infected with *Schistosoma mansoni*. Detection of antigen in serum and urine and correlation between antigenic concentration and worm burden. *Am. J. Trop. Med. Hyg.* 18: 545-552.
- Goldring O.L., J.A. Clegg, S.R. Smithers and R.J. Terry 1976 Acquisition of human blood group antigens by *Schistosoma mansoni*. *Clin. Exp. Immunol.* 26: 181-187.
- Goldring O.L., J.R. Kusel and S.R. Smithers 1977 *Schistosoma mansoni*: Origin *in vitro* of host-like surface antigens. *Expl. Parasitol.* 43: 82-93.
- Goose J. 1978 Possible role of excretory/secretory products in evasion of host defences in *Fasciola hepatica*. *Nature* 275: 216-217.
- Goose J. and M. Macgregor 1973 Naturally acquired immunity to *Fasciola hepatica* in the rat. *Br. Vet. J.* 129: xlix-lii.
- Gorrell M.D. 1977 Antigenic analysis of *Fasciola hepatica*. Honours Thesis, Australian National University.
- Gorrell M.D. and M.J. Howell 1981 A solid phase radioimmunoassay for the detection of antibodies to *Fasciola hepatica*. *Z. Parasitenkd.* Submitted for publication.

- Gorrell M.D. and M.J. Howell 1982 Monoclonal antibodies to *Fasciola hepatica*. In preparation.
- Götze D. and H.P. Vollmers 1979 Reactivity of monoclonal antibodies specific for H-2 antigenic determinants with cells of wild mice. *Immunol. Rev.* 47: 207-218.
- Goudswaard J., J.A. Van Der Donk, A. Noorizij, R.H. Van Dam and J.P. Vaerman 1978 Protein A reactivity of various mammalian immunoglobulins. *Scand. J. Immunol.* 8: 21-28.
- Greene G.L., F.W. Ftich and E.V. Jensen 1980 Monoclonal antibodies to estrophilin: Probes for the study of estrogen receptors. *Proc. Natl. Acad. Sci.* 77: 157-161.
- Greene G.L., C. Nolan, J.P. Engler and E.V. Jensen 1980 Monoclonal antibodies to human estrogen receptor. *Proc. Natl. Acad. Sci.* 77: 5115-5119.
- Gregory M.W. 1979 The globule leukocyte and parasitic infection - a brief history. *Vet. Bull.* 49: 821-827.
- Gross R.L. and P.M. Newberne 1980 Role of nutrition in immunologic function. *Physiol. Rev.* 60: 188-302.
- Grove D.I. and R.H. Civil 1978 *Trichinella spiralis*: Effects on the host-parasite relationship in mice of BCG (attenuated *Mycobacterium bovis*). *Expl. Parasitol.* 44: 181-189.
- Grove D.I., A.A.F. Mahmoud and K.S. Warren 1977 Eosinophils and resistance to *Trichinella spiralis*. *J. Exp. Med.* 145: 755-759.

- Gundlach J.L. 1971a Studies on the serologic activity of *Fasciola hepatica* L. antigens. *Acta Parasitol. Pol.* 19: 9-47.
- Gundlach J.L. 1971b A study of the phenomena of immunity in the course of experimental fascioliasis in rabbits. *Acta Parasitol. Pol.* 19: 285-306.
- Gupta S. and R.A. Good (Eds) 1979a Cellular, Molecular and Clinical Aspects of Allergic Disease: Comprehensive Immunology. Plenum Medical, New York.
- Gupta S. and R.A. Good 1979b Lymphocytes: 1979. *Henry Ford Hosp. Med. J.* 27: 224-235.
- Hajdu E., I. Matskasi and S. Juhasz 1979 *Fasciola hepatica* L. (1758): Studies on protease and protease inhibitor activity. *Parasitol. Hung.* 12: 21-30.
- Hall R.F. and B.Z. Lang 1978 The development of an experimental vaccine against *Fasciola hepatica* in cattle. *Proc. U.S. Animal Health Assoc.* p. 56-60.
- Hammerberg B. and J.F. Williams 1978 Interaction between *Taenia taeniaeformis* and the complement system. *J. Immunol.* 120: 1033-1038.
- Hammerberg B., A.J. Musoke and J.F. Williams 1977 Activation of complement by hydatid cyst fluid in *Echinococcus granulosus*. *J. Parasitol.* 63: 327-331.
- Hammerberg B., C. Dangler and J.F. Williams 1980 *Taenia taeniaeformis*: Chemical composition of parasite factors affecting coagulation and complement cascades. *J. Parasitol.* 66: 569-576.

- Hämmerling G.J., M. Reth, H. Lemke, J. Hewitt, I. Melchers and K. Rajewsky 1978 Fusion of myelomas and T lymphomas with immunocytes. *Coll. Prot. Biol. Fluids* XXV: 551-558.
- Handman E. and J.S. Remington 1980 Serological and immunochemical characterisation of monoclonal antibodies to *Toxoplasma gondii*. *Immunology* 40: 579-587.
- Handman E., J.W. Goding and J.S. Remington 1980 Detection and characterization of membrane antigens of *Toxoplasma gondii*. *J. Immunol.* 124: 2578-2583.
- Hanna R.E.B. 1980a *Fasciola hepatica*: Glycocalyx replacement in the juvenile as a possible mechanism for protection against host immunity. *Expl. Parasitol.* 50: 103-114.
- Hanna R.E.B. 1980b *Fasciola hepatica*: An immunofluorescent study of antigenic changes in the tegument during development in the rat and the sheep. *Expl. Parasitol.* 50: 155-170.
- Hanna R.E.B. 1980c *Fasciola hepatica*: Autoradiography of protein synthesis, transport and secretion by the tegument. *Expl. Parasitol.* 50: 297-304.
- Hanna R.E.B and L.T. Threadgold 1976 *Fasciola hepatica*: Stereological analysis of effects of certain metabolic inhibitors on synthesis of secretory bodies by the tegument of tissue slices. *Expl. Parasitol.* 39: 106-114.
- Hanna R.E.B. and W. Jura 1977 Antibody response of calves to a single infection of *Fasciola gigantica* determined by an indirect fluorescent antibody technique. *Res. Vet. Sci.* 22: 339-342.

- Hansburg D., B. Clevinger, R.M. Perlmutter, R. Griffith, D.E. Briles and J.M. Davie 1979 Analysis of the diversity of murine antibodies to α (1 \rightarrow 3) dextran. In: B. Pernis and H. Vogel *Cells of Immunoglobulin Synthesis*. Academic Press, N.Y. p. 295-308.
- Harness E., D.L. Hughes and T.G. Doy 1976 The demonstration of pre-hepatic immune response to *Fasciola hepatica* in the mouse. *Int. J. Parasitol.* 6: 15-17.
- Harness E., T.G. Doy and D.L. Hughes 1977 The early migratory behaviour of young *Fasciola hepatica* in sensitized mice. *Int. J. Parasitol.* 7: 51-54.
- Haroun E.M., J.A. Hammond and M.M.H. Sewell 1980a Resistance to *Fasciola hepatica* in rats and rabbits following implantation of adult flukes contained in diffusion chambers. *Res. Vet. Sci.* 29: 310-314.
- Haroun E.M., J.A. Hammond and M.M.H. Sewell 1980b Resistance to *Fasciola hepatica* in rats and rabbits following sensitising infection and treatment. *Res. Vet. Sci.* 28: 377-379.
- Harrison L.J.S. 1978 The detection of *Taenia saginata* infection in cattle by the enzyme-linked immunosorbent assay (ELISA). *Trans. R. Soc. Trop. Med. Hyg.* 72: 642.
- Harwell L., B. Skidmore, P. Marrack and J. Kappler 1980 Concanavalin A inducible interleukin 2-producing T cell hybridoma. *J. Exp. Med.* 152: 893-904.

- Hayes T.J. 1978 Further evidence for the early expression of immunity to *Fasciola hepatica* in rats. *J. Parasitol.* 64: 374-376.
- Hayes T.J. and M. Mitrovic 1977 The early expression of protective immunity to *Fasciola hepatica* in rats. *J. Parasitol.* 63: 584-587.
- Hayes T.J., J. Bailer and M. Mitrovic 1972 Immunity in rats to superinfection with *Fasciola hepatica*. *J. Parasitol.* 58: 1103-1105.
- Hayes T.J., J. Bailer and M. Mitrovic 1973 Immunity to *Fasciola hepatica* in rats: The effect of two different levels of primary exposure on superinfection. *J. Parasitol.* 59: 810-812.
- Hayes T.J., J. Bailer and M. Mitrovic 1974a Acquired immunity and age resistance in rats with chronic fascioliasis. *J. Parasitol.* 60: 247-250.
- Hayes T.J., J. Bailer and M. Mitrovic 1974b Serum transfer of immunity to *Fasciola hepatica* in rats. *J. Parasitol.* 60: 722-723.
- Hayes T.J., J. Bailer and M. Mitrovic 1974c Studies on the serum transfer of immunity to *Fasciola hepatica* in the rat. *J. Parasitol.* 60: 930-934.
- Hayes, T.J., J. Bailer and M. Mitrovic 1975 Acquired immunity to *Fasciola hepatica* in splenectomised rats. *Res. Vet. Sci.* 19: 86-87.

- Hayunga E.G., K.D. Murrell, D.W. Taylor and W.E. Vannier 1979
Isolation and characterization of surface antigens from
Schistosoma mansoni. I. Evaluation of techniques for
radioisotope labelling of surface proteins from adult worms.
J. Parasitol. 65: 488-496.
- Healy, G.R. 1955 Studies on immunity to *Fasciola hepatica* in rabbits.
J. Parasitol. 41: Suppl. 25.
- Hengartner H., A.L. Luzzati and M. Schreier 1978 Fusion of *in vitro*
immunized lymphoid cells with X63-Ag8. *Curr. Top. Microbiol.*
Immunol. 81: 92-99.
- Higgins T.J.V., P.B. Goodwin and P.R. Whitfield 1976 Occurrence of
short particles in beans infected with the cowpea strain of TMV.
II. Evidence that short particles contain the cistron for coat
protein. *Virology* 71: 486-497.
- Higgins T.J.V. and D. Spencer 1977 Cell-free synthesis of pea seed
proteins. *Plant Physiol.* 60: 655-661.
- Hillyer G.V. 1975 Use of counterelectrophoresis to detect infections
of *Fasciola hepatica*. *J. Parasitol.* 61: 557-559.
- Hillyer G.V. 1976 Can we vaccinate against schistosomes? *Fed. Proc.*
35: 2568-2571.
- Hillyer G.V. 1979 *Schistosoma mansoni*: Reduced worm burdens in mice
immunized with isolated *Fasciola hepatica* antigens. *Expl.*
Parasitol. 48: 287-295.
- Hillyer G.V. 1980 Isolation of *Fasciola hepatica* tegument antigens.
J. Clin. Microbiol. 12: 695-699.

- Hillyer G.V. and D. Allain 1979 Use of immunologic techniques to detect chemotherapeutic success in infections with *Fasciola hepatica*. III. Comparison of counterelectrophoresis and indirect hemagglutination in infected rabbits. *J. Parasitol.* 65: 960-963.
- Hillyer G.V. and A. Capron 1976 Immunodiagnosis of human fascioliasis by counterelectrophoresis. *J. Parasitol.* 62: 1011-1013.
- Hillyer G.V. and M. Cervoni 1978 Laurell crossed immunoelectrophoresis and affinity chromatography for the purification of a parasite antigen. *J. Immunol. Methods* 20: 385-390.
- Hillyer G.V. and A. Del Llano de Diaz 1976 Use of immunologic techniques to detect chemotherapeutic success in infections of *Fasciola hepatica*. I. Rabbit infections. *Am. J. Trop. Med. Hyg.* 25: 307-311.
- Hillyer G.V. and I. Gomez de Rios 1979 The enzyme-linked immunosorbent assay (ELISA) for the immunodiagnosis of schistosomiasis. *Am. J. Trop. Med. Hyg.* 28: 237-241.
- Hillyer G.V. and C. Manuel 1978 Laurell crossed immunoelectrophoresis and affinity chromatography for the purification of a parasite antigen. *J. Immunol. Methods* 20: 385-390.
- Hillyer G.V. and R.P. Pelley 1980 The major serological antigen (MSA₁) from *Schistosoma mansoni* eggs is a "circumoval" precipitinogen. *Am. J. Trop. Med. Hyg.* 29: 582-585.

- Hillyer G.V. and J.L.R. Ramos 1980 Effect of detergents and acid buffers on the reactivity of *Fasciola hepatica* antigens. *J. Parasitol.* 66: 38-41.
- Hillyer G.V. and L. Sagramoso de Ateca 1979 Immunity in *Schistosoma mansoni* using antigens of *Fasciola hepatica* isolated by concanavalin A affinity chromatography. *Infec. Immun.* 26: 802-807.
- Hillyer G.V. and N. Santiago de Weil 1977 Partial purification of *Fasciola hepatica* antigen for the immunodiagnosis of fascioliasis in rats. *J. Parasitol.* 63: 430-433.
- Hillyer G.V. and N. Santiago de Weil 1979 Use of immunologic techniques to detect chemotherapeutic success in infections with *Fasciola hepatica*. II. The enzyme linked immunosorbent assay in infected rats and rabbits. *J. Parasitol.* 65: 680-684.
- Hillyer G.V. and N. Santiago de Weil 1981 Serodiagnosis of experimental fascioliasis by immunoprecipitation tests. *Int. J. Parasitol.* 11: 71-78.
- Hillyer G.V., A. Del Llano de Diaz and M. Garcia-Blanco 1975 A new approach to the study of immunity to schistosomes. *Bol. Asoc. Med. Puerto Rico* 67: 381-385.
- Hillyer G.V., N.H. Almenas and W.B. Knight 1976 Circulating antigens, antibodies and immunoglobulins in the serum of humans with schistosomiasis mansoni. *Bol. Asoc. Med. Puerto Rico* 68: 128-132.

Hillyer G.V., M. Garcia-Blanco and A. Del Llano de Diaz 1976

A novel approach for protection to schistosomiasis using antigens of *Fasciola hepatica*. *Fed. Proc.* 35: 752.

Hillyer G.V., A. Del Llano de Diaz and C.N. Reyes 1977 *Schistosoma*

mansoni: Acquired immunity in mice and hamsters using antigens of *Fasciola hepatica*. *Expl. Parasitol.* 42: 348-355.

Hillyer G.V., R.P. Pelley and A. Del Llano de Diaz 1979 Solubilization

of antigens of *Fasciola hepatica* which react with antibodies to *Schistosoma mansoni*. *J. Parasitol.* 65: 55-60.

Horibata K. and A.W. Harris 1970 Mouse myelomas and lymphomas in

culture. *Expl. Cell Res.* 60: 61-77.

Houba V. 1979 Experimental renal disease due to schistosomiasis.

Kidney Internat. 16: 30-43.

Houba V. 1980 Schistosomiasis, In: V. Houba *Immunological*

Investigation of Tropical Parasitic Diseases. Churchill

Livingston, Edinburgh, p. 130-147.

Houba V., D.K. Koech, R.F. Sturrock, A.E. Butterworth, J.R. Kusel and

A.A.F. Mahmoud 1976 Soluble antigens and antibodies in sera from baboons infected with *Schistosoma mansoni*. *J. Immunol.* 117: 705-707.

Houston W.E., C.E. Pedersen Jr., F.E. Cole Jr. and R.O. Spertzel

1974 Effects of antigen-antibody complexes on the primary immune response in rhesus monkeys. *Infect. Immun.* 10: 437-442.

Howard J.C. and J.R.F. Corvalan 1979 Demonstration of MHC-specific

haemolytic plaque-forming cells. *Nature* 278: 449-451.

- Howard J.C., G.W. Butcher, G. Galfre, C. Milstein and C.P. Milstein
1979 Monoclonal antibodies as tools to analyse the serological and genetic complexities of major transplantation antigens. *Immunol. Rev.* 47: 139-174.
- Howard G.C., S.M. Abmayr, L.A. Shinefeld, V.L. Sato and S.C. Elgin
1981 Monoclonal antibodies against a specific nonhistone chromosomal protein of *Drosophila melanogaster* associated with active genes. *J. Cell Biol.* 88: 219-225.
- Howard R.J., C.B. Chapman and G.F. Mitchell 1980 A difference in surface proteins of *Fasciola hepatica* larvae from intact and nude mice. *Aust. J. Exp. Biol. Med. Sci.* 58: 201-205.
- Howell M.J. 1973 Localisation of proteolytic activity in *Fasciola hepatica*. *J. Parasitol.* 59: 454-456.
- Howell M.J. 1979 Vaccination of rats against *Fasciola hepatica*. *J. Parasitol.* 65: 817-819.
- Howell M.J. 1981 An approach to the production of helminth antigens *in vitro*: The formation of hybrid cells between *Fasciola hepatica* and a rat fibroblast cell line. *Int. J. Parasitol.* 11: 235-242.
- Howell M.J. and R.M. Sandeman 1979 *Fasciola hepatica*: Some properties of a precipitate which forms when metacercariae are cultured in immune rat serum. *Int. J. Parasitol.* 9: 41-45.
- Howell M.J., R.M. Sandeman and G.R. Rajasekariah 1977 *In vivo* and *in vitro* studies of the effects of immune rat serum on *Fasciola hepatica*. *Int. J. Parasitol.* 7: 367-371.

- Howell R.M. 1966 Collagenase activity of immature *Fasciola hepatica*. *Nature* 209: 713-714.
- Hrzenjak T., V. Muic and I. Erlich 1979 Komparativna studija poliheksozamin-ceramidnog kompleksa izoliranog iz *Echinococcus granulosus* larvae i *Fasciola hepatica*. *Veter. Arch. Zagreb* 49: 21-32.
- Hsu S.Y.L., H.F. Hsu, P. Isacson and H.F. Cheng 1977 *In vitro* schistosomulocidal effect of immune serum and eosinophils, neutrophils and lymphocytes. *J. Retic. Soc.* 21: 153-162.
- Hughes D.L. 1962a Observations on the immunology of *Fasciola hepatica* infections in mice and rabbits. *Parasitology* 52: 4 p.
- Hughes D.L. 1962b Reduction of the pathogenicity of *Fasciola hepatica* in mice by X irradiation. *Nature* 193: 1093-1094.
- Hughes D.L. and E. Harness 1973a Attempts to demonstrate a host antigen effect by the experimental transfer of adult *Fasciola hepatica* into recipient animals immunized against the donor. *Res. Vet. Sci.* 14: 151-154.
- Hughes D.L. and E. Harness 1973b The experimental transfer of immature *Fasciola hepatica* from donor mice and hamsters to rats immunised against the donors. *Res. Vet. Sci.* 14: 220-222.
- Hughes D.L., E. Harness and T.G. Doy 1976a The establishment and duration of *Fasciola hepatica* infections in two strains of rats and the development of acquired resistance. *Res. Vet. Sci.* 20: 207-211.

- Hughes D.L., J.C. Anderson and E. Harness 1976b *Fasciola hepatica*:
The effect of challenge by the subcutaneous or intraperitoneal
route with living adult flukes in sensitized rats. *Expl. Parasitol.*
40: 355-362.
- Hughes D.L., E. Harness and T.G. Doy 1977 Loss of ability to kill
Fasciola hepatica in sensitized rats. *Nature* 267: 517-518.
- Hughes D.L., E. Harness and T.G. Doy 1978 Failure to demonstrate
resistance in goats, sheep and cattle to *Fasciola hepatica* after
infection with *Cysticercus tenuicollis*. *Res. Vet. Sci.* 25: 356-359.
- Hughes D.L., E. Harness and T.G. Doy 1979 Preliminary observations
on *Fasciola hepatica* in normal and sensitized nude rats.
Parasitology 79: xxxi.
- Hughes D.L., E. Harness and T.G. Doy 1981 The different stages of
Fasciola hepatica capable of inducing immunity and the
susceptibility of various stages to immunological attack in the
sensitized rat. *Res. Vet. Sci.* 30: 93-98.
- Hunter W.M. and F.C. Greenwood 1962 Preparation of iodine-131
labelled human growth hormone of high specific activity. *Nature*
194: 495-496.
- Hutt M.S.R. 1980 Renal disease in a tropical environment. *Trans.*
R. Soc. Trop. Med. 74: 17-21.
- Iacona A., C. Pini and G. Vicari 1980 Enzyme-linked immunosorbent
assay (ELISA) in the serodiagnosis of hydatid disease. *Am. J.*
Trop. Med. Hyg. 29: 95-102.

- Inganas M., S.G.O. Johansson and H.H. Bennich 1980 Interaction of human polyclonal IgE and IgG from different species with protein A from *Staphylococcus aureus*: Demonstration of protein A reactive sites located in the Fab₂ fragment of human IgG. *Scand. J. Immunol.* 12: 23-31.
- Inglis J.R. 1980 Monoclonal antibodies - their production and uses. *Trends Neur.* 3: 192-193.
- INSERM 1978 Immunity in Parasitic Diseases. Eds A. Capron, P-H. Lambert, B. Ogilvie and P. Pery. Volume 72, Paris.
- Irving, D.O. and M.J. Howell 1982a Preparation and *in vitro* translation of mRNA from *Fasciola hepatica*. *Mol. Biochem. Parasitol.* In press.
- Irving D.O. and M.J. Howell 1982b Characterization of some excretory/secretory antigens of *Fasciola hepatica*. *Parasitology*. Submitted for publication.
- Ishikawa H. and K. Saito 1980 Congenitally athymic nude (nu/nu) mice have Thy-1-bearing immunocompetent helper T cells in their peritoneal cavity. *J. Exp. Med.* 151: 965-968.
- Izant J.G. and J.R. McIntosh 1980 Microtubule-associated proteins: A monoclonal antibody to MAP2 binds to differentiated neurons. *Proc. Natl. Acad. Sci.* 77: 4741-4745.
- James S.L. and A. Sher 1980 Immune mechanisms that stimulate mouse leukocyte migration in response to schistosomula of *Schistosoma mansoni*. *J. Immunol.* 124: 1837-1844.

Janossy G., N. Tidman, E.S. Papageorgiou, P.C. Kung and G. Goldstein

1981 Distribution of T lymphocyte subsets in the human bone marrow and thymus: An analysis with monoclonal antibodies.

J. Immunol. 126: 1608-1613.

Jarrett E.E.E. 1972 Potentiation of reaginic (IgE) antibody to

ovalbumin in the rat following sequential trematode and nematode infections. *Immunology* 22: 1099-1101.

Jarrett E.E.E. and G.M. Urquhart 1971 The immune response to

nematode infections. *Int. Rev. Trop. Med.* 4: 53-96.

Jarrett E.E.E. and H. Bazin 1974 Elevation of total serum IgE in

rats following helminth parasite infection. *Nature* 251: 613-614.

Jerne N.K., C. Henry, A.A. Nordin, H. Fuji, A.M.C. Koros and I.

Lefkovits 1974 Plaque forming cells: Methodology and theory.

Transplant. Rev. 18: 130-191.

Johnson A.M., P.J. McNamara, S.H. Neoh, P.J. McDonald and H. Zola

1981 Hybridomas secreting monoclonal antibody to *Toxoplasma gondii*. *Aust. J. Exp. Biol. Med. Sci.* 59: 303-306.

Jong E.C., W.R. Henderson and S.J. Klebanoff 1980 Bactericidal

activity of eosinophil peroxidase. *J. Immunol.* 124: 1378-1382.

Jong E.C., A.A.F. Mahmoud and S.J. Klebanoff 1981 Peroxidase-

mediated toxicity to schistosomula of *Schistosoma mansoni*.

J. Immunol. 126: 468-471.

Joseph M., J-P. Dessaint and A. Capron 1977 Characteristics of

macrophage activation by IgE immune complexes. *Cell Immunol.* 34:

247-258.

- Joseph M., A. Capron, A.E. Butterworth, R.F. Sturrock and V. Houba
1978 Cytotoxicity of human and baboon mononuclear phagocytes against schistosomula *in vitro*: Induction by immune complexes containing IgE and *Schistosoma mansoni* antigens. *Clin. Exp. Immunol.* 33: 48-56.
- Juy D., P. Legrain, P-A. Cazenave and G. Buttin 1979 A new rapid rosette-forming cell micromethod for the detection of antibody-synthesizing hybridomas. *J. Immunol. Methods* 30: 269-275.
- Kagan I.G. 1979 Diagnostic, epidemiologic and experimental parasitology: Immunologic aspects. *Am. J. Trop. Med. Hyg.* 28: 429-439.
- Kagan I.G. 1980 Other trematodes and cestodes. In: V. Houba *Immunological Investigations of Tropical Parasitic Diseases*. Churchill Livingston, Edinburgh. p. 148-156.
- Kano K. and F. Milgrom 1980 Immune complex diseases. *Vox. Sang.* 38: 121-137.
- Kano K., B.B. Knowles, H. Koprowski and F. Milgrom 1972 HL-A antigens on man-mouse hybrid cells. *Eur. J. Immunol.* 2: 198-202.
- Kassis A.I. and C.E. Tanner 1976 The role of complement in hydatid disease: *In vitro* studies. *Int. J. Parasitol.* 6: 25-35.
- Kassis A.I. and C.E. Tanner 1977 *Echinococcus multilocularis*: Complement's role *in vitro* in hydatid disease. *Expl. Parasitol.* 43: 390-395.

- Kassis A.I., M. Aikawa and A.A.F. Mahmoud 1979a Mouse antibody-dependent eosinophil and macrophage adherence and damage to schistosomula of *Schistosoma mansoni*. *J. Immunol.* 122: 398-405.
- Kassis A.I., K.S. Warren and A.A.F. Mahmoud 1979b Antibody-dependent complement-mediated killing of schistosomula in intraperitoneal diffusion chambers in mice. *J. Immunol.* 123: 1659-1662.
- Katz D.H., T.E. Bechtold and A. Altman 1980 Construction of T cell hybridomas secreting allogeneic effector factor. *J. Exp. Med.* 152: 956-966.
- Katzmann J.A., M.E. Nesheim, L.S. Hibbard and K.G. Mann 1981 Isolation of functional human coagulation Factor V by using a hybridoma antibody. *Proc. Natl. Acad. Sci.* 78: 162-166.
- Kay A.B. 1978 The eosinophil leucocyte: Formation, function and fate. *INSERM* 72: 201-216.
- Kay A.B., H.S. Shin and K.F. Austen 1973 Selective attraction of eosinophils and synergism between eosinophil chemotactic factor of anaphylaxis (ECF-A) and a fragment cleaved from the fifth component of complement (C5a). *Immunology* 24: 969-976.
- Kazacos K.R. 1976 Immunization of mice against *Mesocostoides corti* by subcutaneous inoculation of living tetrathyridia. *J. Parasitol.* 62: 161-163.
- Kazura J.W. and D.I. Grove 1978 Stage-specific antibody-dependent eosinophil-mediated destruction of *Trichinella spiralis*. *Nature* 274: 588-589.

- Kearney J.F., A. Radbruch, B. Liesegang and K. Rajewsky 1979 A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. *J. Immunol.* 123: 1548-1550.
- Kelly B.S., J.G. Levy and L. Sikora 1979 The use of the enzyme-linked immunosorbent assay (ELISA) for the detection and quantification of specific antibody from cell cultures. *Immunology* 37: 45-52.
- Kelly J.D. and N.J. Campbell 1979 The effect of route of infection on acquired resistance to *Fasciola hepatica* in the rat and sheep. *Res. Vet. Sci.* 27: 205-209.
- Kelly J.D., N.J. Campbell and J.K. Dineen 1980 The role of the gut in acquired resistance to *Fasciola hepatica* in the rat. *Vet. Parasitol.* 6: 359-367.
- Kelsoe G., M. Reth and K. Rajewsky 1980 Control of idiotype expression by monoclonal anti-idiotypic antibodies. *Immunol. Rev.* 52: 75-88.
- Kemp D.J. and A.F. Cowman 1981 Direct immunoassay for detecting *Escherichia coli* colonies that contain polypeptides encoded by cloned DNA segments. *Proc. Natl. Acad. Sci.* 78: 4520-4524.
- Kemp W.M., R.T. Damian, N.D. Greene and W.B. Lushbaugh 1976 Immunocytochemical localisation of mouse alpha 2-macroglobulin-like antigenic determinants on *Schistosoma mansoni* adults. *J. Parasitol.* 62: 413-419.

- Kemp W.M., P.R. Brown, S.C. Merritt and R.E. Miller 1980
Tegument-associated antigen modulation by adult male *Schistosoma mansoni*. *J. Immunol.* 124: 806-811.
- Kendall S.B. 1967 Host specificity as evidenced by species of *Fasciola*. *Helminthologia* 8: 223-233.
- Kendall S.B. and I.J. Sinclair 1971 Barriers to reinfection with *Fasciola hepatica* in the rabbit. *Res. Vet. Sci.* 12: 74-79.
- Kendall S.B., N. Herbert, J.W. Parfitt and M.A. Peirce 1967
Resistance to reinfection with *Fasciola hepatica* in rabbits. *Expl. Parasitol.* 20: 242-247.
- Kendall S.B., I.J. Sinclair, G. Everett and J.W. Parfitt 1978
Resistance to *Fasciola hepatica* in cattle I. Parasitological and serological observations. *J. Comp. Pathol.* 88: 115-122.
- Kennett R.H. 1979 Cell fusion. *Methods Enz.* 58: 345-359.
- Kennett R.H., K.A. Denis, A.S. Tung and N.R. Klinman 1978 Hybrid plasmacytoma production: Fusions with adult spleen cells, monoclonal spleen fragments, neonatal spleen cells and human spleen cells. *Curr. Top. Microbiol. Immunol.* 81: 77-91.
- Kennett R.H., T.J. McKearn and K.B. Bechtol (Eds) 1980 Monoclonal Antibodies. Plenum Press, New York.
- Kent N.H. 1960 Isolation of specific antigens from *Ascaris lumbricoides* (var. suum). *Expl. Parasitol.* 10: 313-323.
- Kerbal R.S. 1980 Increased sensitivity of rosetting assay for Fc receptors obtained by using non-haemagglutinating monoclonal antibodies. *J. Immunol. Methods* 34: 1-10.

- Kerr K.B. and O.L. Petkovich 1935 Active immunity in rabbits to the liver fluke, *Fasciola hepatica*. *J. Parasitol.* 21: 319-320.
- Kessler S.W. 1975 Rapid isolation of antigens from cells with a staphylococcal protein A-antibody adsorbent: Parameters of the interaction of antibody-antigen complexes with protein A. *J. Immunol.* 115: 1617-1624.
- Kessler S.W. 1976 Cell membrane antigen isolation with the staphylococcal protein A-antibody adsorbent. *J. Immunol.* 117: 1482-1490.
- Kiessling R. and H. Wigzell 1979 An analysis of the murine NK cell as to structure, function and biological relevance. *Immunol. Rev.* 44: 165-208.
- Kim K.J., D.W. Taylor, C.B. Evans and R. Asofsky 1980 Radioimmunoassay for detecting antibodies against murine malarial parasite antigens: Monoclonal antibodies recognising *Plasmodium yoelii* antigens. *J. Immunol.* 125: 2565-2569.
- Kincade P.W., G. Lee, L. Sun and T. Watanabe 1981 Monoclonal rat antibodies to murine IgM determinants. *J. Immunol. Methods* 42: 17-26.
- Klinman N.R. 1969 Antibody with homogeneous antigen binding produced by splenic foci in organ culture. *Immunochem.* 6: 757-759.
- Klinman N.R. 1971 Purification and analysis of 'monofocal' antibody. *J. Immunol.* 106: 1345-1352.

- Klinman N.R., A.R. Pickard, N.H. Sigal, P.J. Gearhart, E.S. Metcalf and S.K. Pierce 1976 Assessing B cell diversification by antigen receptor and precursor cell analysis. *Ann. Immunol. Paris* 127C: 489-502.
- Knight R.A. 1978 Experimental cross infections of *Fasciola hepatica* in lambs and calves. *J. Parasitol.* 64: 601-605.
- Knight R.A. 1980a Response of lambs to challenge infections after repeated inoculations with *Fasciola hepatica* cysts. *Proc. Helminthol. Soc. Wash.* 47: 186-191.
- Knight R.A. 1980b Relationship of globule leukocytes to infections with *Fasciola hepatica* in sheep. *J. Parasitol.* 66: 844-845.
- Knopf P.M. and M. Soliman 1980 Effects of host endocrine gland removal on the permissive status of laboratory rodents to infection by *Schistosoma mansoni*. *Int. J. Parasitol.* 10: 197-204.
- Knutton S. and C.A. Pasternak 1979 The mechanism of cell-cell fusion. *Trends Bioc. Sci.* 4: 220-223.
- Köhler G. 1979 Fusion of lymphocytes. In: I. Lefkovitz and B. Pernis *Immunological Methods*. Academic Press, N.Y., p. 391-395.
- Köhler G. and C. Milstein 1975 Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256: 495-497.
- Köhler G. and C. Milstein 1976 Derivation of specific antibody-producing tissue culture and tumour lines by cell fusion. *Eur. J. Immunol.* 6: 511-519.

- Köhler G., S.C. Howe and C. Milstein 1976 Fusion between immunoglobulin-secreting and nonsecreting myeloma cell lines. *Eur. J. Immunol.* 6: 292-295.
- Köhler G., T. Pearson and C. Milstein 1977 Fusion of T and B cells. *Som. Cell Genet.* 3: 303-312.
- Koie M., P. Nansen and N.O. Christensen 1977 Stereoscan studies of rediae, cercariae, cysts, excysted metacercariae and migratory stages of *Fasciola hepatica*. *Z. Parasitenkd.* 54: 289-297.
- Kondo K., H. Yoshimura, Y. Ohnishi, N. Akao, N. Tsubota, T. Susuki and S. Ishigooka 1980 Immunological studies on diphyllbothriasis, with special reference to latex-agglutination and enzyme-linked immunosorbent assay (ELISA). *Jap. J. Parasitol.* 29: 55-60.
- Koprowski H., W. Gerhard and C.M. Croce 1977 Production of antibodies against influenza virus by somatic cell hybrids between mouse myeloma and primed spleen cells. *Proc. Natl. Acad. Sci.* 74: 2985-2988.
- Koprowski H., W. Gerhard, T. Wiktor, J. Martinis, M. Shander and C.M. Croce 1978 Anti-viral and anti-tumour antibodies produced by somatic cell hybrids. *Curr. Top. Microbiol. Immunol.* 81: 8-19.
- Korach S. and J. Benex 1966a A lipoprotein antigen in *Fasciola hepatica*. I. Isolation, physical and chemical data. *Expl. Parasitol.* 19: 193-198.
- Korach S. and J. Benex 1966b A lipoprotein antigen in *Fasciola hepatica*. II. Immunological and immunochemical properties. *Expl. Parasitol.* 19: 199-205.

- Kozar M. 1974 The effect of immunization of the final host with intermediate host antigens on the development of *Fasciola hepatica* and the course of fascioliasis in rats. *Acta Parasitol. Pol.* 22: 473-483.
- Kusel J.R. and P.E. Mackenzie 1975 The measurement of the relative turnover rates of proteins of the surface membranes and other fractions of *Schistosoma mansoni* in culture. *Parasitology* 71: 261-273.
- Kusel J.R., P.E. Mackenzie and D.J. McLaren 1975 The release of membrane antigens into culture by adult *Schistosoma mansoni*. *Parasitology* 71: 247-259.
- Kusel J.R., A. Sher, H. Perez, J.A. Clegg and S.R. Smithers 1975 Use of radioactive isotopes in the study of specific schistosome membrane antigens. In: *Isotopes and Radiation in Parasitology IV*. International Atomic Energy Agency, Vienna, p. 127-143.
- Lachmann P.J., R.G. Olroyd, C. Milstein and B.W. Wright 1980 Three rat monoclonal antibodies to human complement C3. *Immunology* 41: 503-516.
- Laissue J., H. Cottier, M.W. Hess and R.D. Stoner 1971 Early and enhanced germinal centre formation and antibody responses in mice after primary stimulation with antigen - isologous antibody complexes as compared with antigen alone. *J. Immunol.* 107: 822-829.
- Lajtha L.G. 1980 Strategic reserves. *Blood Cells* 6: 381-389.
- Lalic R., K. Cuperlovic and M. Movsesijan 1976 Studies on immunogenicity of secretory/excretory antigen of *Fasciola hepatica*. *Acta Vet. Beograd* 26: 69-77.

- Lambden P.R. and P.J. Watt 1978 A solid phase radioimmunoassay on hydrophobic membrane filters: Detection of antibodies to gonococcal surface antigens. *J. Immunol. Methods* 20: 277-286.
- Lang B.Z. 1967 Host-parasite relationships of *Fasciola hepatica* in the white mouse. II. Studies on acquired immunity. *J. Parasitol.* 53: 21-30.
- Lang B.Z. 1968 Acquired immunity to *Fasciola hepatica* in the laboratory white mouse. *J. Trop. Med. Hyg.* 17: 561-567.
- Lang B.Z. 1974 Host-parasite relationships of *Fasciola hepatica* in the white mouse. VI. Studies on the effects of immune and normal sera on the viability of young worms transferred to normal recipients. *J. Parasitol.* 60: 925-929.
- Lang B.Z. 1976 Host-parasite relationships of *Fasciola hepatica* in the white mouse. VII. Effects of anti-worm incubate sera on transferred worms and successful vaccination with a crude incubate antigen. *J. Parasitol.* 62: 232-236.
- Lang B.Z. and R.F. Hall 1977 Host parasite relationships of *Fasciola hepatica* in the white mouse VIII. Successful vaccination with culture incubate antigens and antigens from sonic disruption of immature worms. *J. Parasitol.* 63: 1046-1049.
- Lang B.Z., J.E. Larsh, N.F. Weatherly and H.T. Goulson 1967 Demonstration of immunity to *Fasciola hepatica* in recipient mice given peritoneal exudate cells. *J. Parasitol.* 53: 208-209.
- Langone J.J. 1978 ^{125}I protein A: A tracer for general use in immunoassay. *J. Immunol. Methods* 24: 269-285.

- Langone J.J. 1980 ^{125}I -labelled protein A as a general tracer in immunoassay: Suitability of goat and sheep antibodies. *J. Immunol. Methods* 34: 93-106.
- Langone J.J., M.D.P. Boyle and T. Boros 1977 ^{125}I Protein A: Applications to the quantitative determination of fluid phase and cell-bound IgG. *J. Immunol. Methods* 18: 281-293.
- Laskov R., K.J. Kimm and R. Asofsky 1978 Induction of IgM secretion by fusing murine B lymphoma with myeloma cells. *Curr. Top. Microbiol. Immunol.* 81: 173-175.
- Laver W.G., G.M. Air, R.G. Webster, W. Gerhard, C.W. Ward and T.A.A. Dopheide 1979 Antigenic drift in type A influenza virus: Sequence differences in the haemmagglutinin of Hong Kong (H3N2) variants selected with monoclonal hybridoma antibodies. *Virology* 98: 226-237.
- Le Clair K., A. Van Agthoven and C. Terhorst 1981 A rapid method for isolation of antigenically active human cell surface antigens associated with β_2 -microglobulin using a monoclonal antibody. *J. Immunol. Methods* 41: 137-144.
- Ledbetter J.A. and L.A. Herzenberg 1979 Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol. Rev.* 47: 63-90.
- Ledbetter J.A., R.V. Rouse, S. Micklem and L.A. Herzenberg 1980 T cell subsets defined by expression of Lyt-1,2,3 and Thy-1 antigens. *J. Exp. Med.* 152: 280-295.

- Lehner R.P. and M.M.H. Sewell 1979 Attempted immunization of laboratory animals with metabolic antigens of *Fasciola hepatica*. *Vet. Sci. Comm.* 2: 337-340.
- Lehner R.P. and M.M.H. Sewell 1980 A study of the antigens produced by adult *Fasciola hepatica* maintained *in vitro*. *Parasite Immunol.* 2: 99-109.
- Lehtonen O.P. and M.K. Viljanen 1980 Antigen attachment in ELISA. *J. Immunol. Methods* 34: 61-70.
- Leininger R.I., C.W. Cooper, M.M. Epstein, R.D. Falb and G.A. Grode 1966 Nonthrombogenic plastic surfaces. *Science* 152: 1625-1626.
- Leiper J.W.G. 1938 The longevity of *Fasciola hepatica*. *J. Helminthol.* 16: 173-176.
- Lemke H., G.J. Hämmerling, C. Höhmann and K. Rajewsky 1978 Hybrid cell lines secreting monoclonal antibody specific for major histocompatibility antigens of the mouse. *Nature* 271: 249-251.
- Lemke H., G.J. Hämmerling and U. Hämmerling 1979 Fine specificity analysis with monoclonal antibodies of antigens controlled by the major histocompatibility complex and by the Qa/TL region in mice. *Immunol. Rev.* 47: 175-206.
- Levine D.M., G.V. Hillyer and S.I. Flores 1980 Comparison of counter-electrophoresis, the enzyme-linked immunosorbent assay, and Kato fecal examination for the diagnosis of fascioliasis in infected mice and rabbits. *Am. J. Trop. Med. Hyg.* 29: 602-608.
- Levy R. and J. Dilley 1977 The *in vitro* antibody response to cell surface antigens. I. The xenogeneic response to human leukemia cells. *J. Immunol.* 119: 387-393.

- Levy R. and J. Dilley 1978 Rescue of immunoglobulin secretion from human neoplastic lymphoid cells by somatic cell hybridisation. *Proc. Natl. Acad. Sci.* 75: 2411-2415.
- Levy R., J. Dilley and L.A. Lampson 1978 Human normal and leukemia cell surface antigens. Mouse monoclonal antibodies as probes. *Curr. Top. Microbiol. Immunol.* 81: 164-169.
- Lewicki J.A., H.J. Brandwein, S.A. Waldman and F. Murad 1980 Purified guanylate cyclase: Characterisation, iodination and preparation of monoclonal antibodies. *J. Cyclic Nuc. Res.* 6: 283-296.
- Liedgens W., R. Gruetzmänn and H.A. Schneider 1980 Highly efficient purification of the labile plant enzyme 5-amino levulinate dehydratase EC-4.2.1.24 by means of monoclonal antibodies. *Z. Naturforsch.* C35: 959-962.
- Lindsley H.B., L.L. Janecek and K. Esser 1981 Composition of circulating immune complexes in experimental African trypanosomiasis. *Fed. Proc.* 40: 1114.
- Ling N.R., G. Stephens, P. Bratt and H.S. Dhaliwal 1979 Attachment of antigens and antibodies to fixed red cells: Their use in rosette and haemagglutination tests; a comparison with fresh red cells. *Mol. Immunol.* 16: 637-642.
- Littlefield J.W. 1964 Selection of hybrids from matings of fibroblasts *in vitro* and their presumed recombinants. *Science* 145: 709.
- Lloyd S. 1981 Progress in immunization against parasitic helminths. *Parasitology* 83: 225-242.

- Locatelli A. and C. Beretta 1969 Detection of aminoacidic N in saline or serum media incubated with *Fasciola hepatica* and proteolytic activity exerted by liverfluke *in vitro*. *Arch. Vet. Ital.* 20: 385-391.
- Locatelli A. and A. Marzo 1969 Serological and immunoelectrophoretic findings in rabbits immunized with somatic antigens of *Fasciola hepatica*. *Arch. Vet. Ital.* 20: 27-31.
- Locatelli A. and C. Paoletti 1969 *Fasciola hepatica* L. homogenate proteolytic activity: Action against different substrates. *Arch. Vet. Ital.* 20: 97-100.
- Locatelli A., and T. Simonic 1974 *Fasciola hepatica* L. and fascioliasis: Physiological, biochemical, pathogenic, immunologic and diagnostic aspects. *Folia Vet. Lat.* 4: 43-70.
- Lotzova E. and K.B. McCredie 1978 Natural killer cells in mice and man and their possible biological significance. *Cancer Immunol. Immunother.* 4: 215-221.
- Lowell G.H., L.F. Smith, M.S. Artenstein, G.S. Nash and R.P. MacDermott 1979 Antibody-dependent cell-mediated antibacterial activity of human mononuclear cells. I. K lymphocytes and monocytes are effective against meningococci in cooperation with human immune sera. *J. Exp. Med.* 150: 127-137.
- Lowry O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall 1951 Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193: 265-275.

- Luben R.A., M.A. Mohler and G.E. Nedwin 1979 Production of hybridomas secreting monoclonal antibodies against the lymphokine osteoclast activating factor. *J. Clin. Invest.* 64: 337-341.
- Lumsden D.R. 1975 Surface ultrastructure and cytochemistry of parasitic helminths. *Expl. Parasitol.* 37: 267-339.
- Lunde M.N. and E.A. Ottesen 1980 Enzyme-linked immunosorbent assay (ELISA) for detecting IgM and IgE antibodies in human schistosomiasis. *Am. J. Trop. Med. Hyg.* 29: 82-85.
- Luzzati A.L., H. Hengartner and M.H. Schreier 1977 Induction of plaque-forming cells in cultured human lymphocytes by combined action of antigen and EB virus. *Nature* 269: 419-420.
- Lyon J.A., J.M. Pratt, R.W. Travis, B.P. Doctor and J.G. Olenick 1981 Use of monoclonal antibody to immunochemically characterize variant-specific surface coat glycoprotein from *Trypanosoma rhodesiense*. *J. Immunol.* 126: 134-137.
- McKearn T.J., F.W. Fitch, D.E. Smilek, M. Sarmiento and F.P. Stuart 1979 Properties of rat anti-MHC antibodies produced by cloned rat-mouse hybridomas. *Immunol. Rev.* 47: 91-116.
- McKenzie M.R., N.L. Warner and G.F. Mitchell 1978 The binding of murine immunoglobulins to staphylococcal protein A. *J. Immunol.* 120: 1495-1496.
- McLaren D.J. and F.J. Ramalho-Pinto 1979 Eosinophil-mediated killing of schistosomes of *Schistosoma mansoni* in vitro: Synergistic effect of antibody and complement. *J. Immunol.* 123: 1431-1438.

- McLaren D.J., F.J. Ramalho-Pinto and S.R. Smithers 1978
Ultrastructural evidence for complement and antibody-dependent damage to schistosomula of *Schistosoma mansoni* by rat eosinophils *in vitro*. *Parasitology* 77: 313-324.
- McLaren M.L., E.G. Long, R.W. Goodgame and J.E. Lillywhite 1979
Application of the enzyme linked immunosorbent assay (ELISA) for the serodiagnosis of *Schistosoma mansoni* infection in St Lucia. *Trans. R. Soc. Trop. Med. Hyg.* 73: 636-639.
- McMaster W.R. and A.F. Williams 1979 Monoclonal antibodies to Ia antigens from rat thymus: Cross reactions with mouse and human and use in purification of rat Ia glycoproteins. *Immunol. Rev.* 47: 117-138.
- McMichael A.J., J.R. Pilch, G. Galfre, D.Y. Mason, J.W. Fabre and C. Milstein 1979 A human thymocyte antigen defined by a hybrid myeloma monoclonal antibody. *Eur. J. Immunol.* 9: 205-210.
- Maddison S.E., I.G. Kagan, F.W. Chandler, D. Gold, G.V. Hillyer, S.B. Slemenda and V. Tsang 1979 Immunization against *Schistosoma mansoni* in rhesus monkeys and the requirement of activation of both cell-mediated and humoral mechanisms. *Infect. Immun.* 25: 237-248.
- Madwar M.A. and A. Voller 1975 Circulating soluble antigens and antibodies in schistosomiasis. *Br. Med. J.* 1: 435-436.
- Mahmoud A.A.F., P.A.S. Peters, R.H. Civil and J.S. Remington 1979
In vitro killing of *Schistosoma mansoni* by BCG and *C. parvum*-activated macrophages. *J. Immunol.* 122: 1655-1657.

- Maki R., J. Kearney, C. Paige and S. Tonegawa 1980 Immunoglobulin gene rearrangement in immature B cells. *Science* 209: 1366-1369.
- Marier R., M. Jansen and V.T. Andriole 1979 A new method for measuring antibody using radiolabelled protein A in a solid-phase radioimmunoassay. *J. Immunol. Methods* 28: 41-49.
- Margulies D.H., W.M. Kuehl and M.D. Scharff 1976 Somatic cell hybridization of mouse myeloma cells. *Cell* 8: 405-415.
- Matossian R.M., M.L. McLaren, C.C. Draper, C.M.P. Bradstreet M.W. Dighero, G.J. Kane, L.M. Mackinlay and M.D. Rickard 1979 The serodiagnosis of human hydatid disease: 2. Additional studies in selected sera using indirect haemagglutination (IHA), enzyme linked immunosorbent assay (ELISA) and defined antigen substrate spheres (DASS). *J. Helminthol.* 53: 287-291.
- Mauel J. 1978 Immunity to protozoan parasites. *INSERM* 72: 127-146.
- Medgyesi G.A., G. Füst, J. Gergely and H. Bazin 1978 Classes and subclasses of rat immunoglobulins: Interaction with the complement system and with staphylococcal protein A. *Immunochemistry* 15: 125-129.
- Meek A.H. and R.S. Morris 1979a The longevity of *Fasciola hepatica* metacercariae encysted on herbage. *Aust. Vet. J.* 55: 58-60.
- Meek A.H. and R.S. Morris 1979b The effect of prior infection with *Fasciola hepatica* on the resistance of sheep to the same parasite. *Aust. Vet. J.* 55: 61-64.
- Melchers F., M. Potter and N.L. Warner 1978 Lymphocyte hybridomas. *Curr. Top. Microbiol. Immunol.* 81: ix-xxiii.

- Mellman I.S. and J.C. Unkeless 1980 Purification of a functional mouse Fc receptor through the use of a monoclonal antibody. *J. Exp. Med.* 152: 1048-1068.
- Mettrick D.F. and R.B. Podesta 1974 Ecological and physiological aspects of helminth-host interactions in the mammalian gastrointestinal canal. *Adv. Parasitol.* 12: 183-287.
- Milgrom F., T. Luszczynski and S. Dubiski 1956 Preparation of antiglobulin sera. *Nature* 177: 329.
- Miller T.J. and H.O. Stone 1978 The rapid isolation of ribonuclease-free immunoglobulin G by protein A-sepharose affinity chromatography. *J. Immunol. Methods* 24: 111-125.
- Milstein C. and E. Lennox 1980 The use of monoclonal antibody techniques in the study of developing cell surfaces. *Curr. Top. Dev. Biol.* 14(II): 1-32.
- Milstein C. 1980 Monoclonal antibodies. *Sci. Amer.* 243: 56-64.
- Minning W. and G. Fuhrmann 1955 Protein-kohlehydrat-und lipoid-fractionen von *Fasciola hepatica* als KBR-antigen. *Z. Tropenmed. Parasitol.* 6: 92-99.
- Minning W. and H. Vogel 1950 Immunobiologische und epidemiologische untersuchen beiz Fallen von menschlicher Fasciolose. *Z. Tropenmed. Parasitol.* 1: 532-553.
- Minning W., J. Newsome and D.L.H. Robinson 1958 Trematoden-stoffwechselproducte als antigene. *Z. Tropenmed. Parasitol.* 9: 335-342.

Mitchell G.B.B., J. Armour, J.G. Ross and W.G. Halliday 1981

Successful passive transfer of resistance to *Fasciola hepatica* infection in rats by immune serum and transfer factor. *Res. Vet. Sci.* 30: 246-247.

Mitchell G.F. 1977 Host protective immunity in parasitic diseases.

Prog. Immunol. III: 675-680.

Mitchell G.F. 1979a Effector cells, molecules and mechanisms in

host-protective immunity to parasites. *Immunology* 38: 209-223.

Mitchell G.F. 1979b Responses to infection with metazoan and

protozoan parasites in mice. *Adv. Immunol.* 28: 451-511.

Mitchell G.F., J.J. Marchalonis, P.M. Smith, W.L. Nicholas and N.L.

Warner 1977 Studies on immune responses to larval cestodes in mice. Immunoglobulins associated with the larvae of *Mesocostoides corti*. *Aust. J. Exp. Biol. Med. Sci.* 55: 187-211.

Mitchell G.F., K.M. Cruise, C.B. Chapman, R.F. Anders and M. Howard

1979 Hybridoma antibody immunoassays for the detection of parasitic infection: Development of a model system using a larval cestode infection in mice. *Aust. J. Exp. Biol. Med. Sci.* 57: 287-302.

Mitchell G.F., K.M. Cruise, E.G. Garcia and R.F. Anders 1981

Hybridoma-derived antibody with immunodiagnostic potential for schistosomiasis japonica. *Proc. Natl. Acad. Sci.* 78: 3165-3169.

Moser G., D.L. Wassom and A. Sher 1980 Studies of the antibody-

dependent killing of schistosomula of *Schistosoma mansoni* employing haptenic target antigens. I. Evidence that the loss in

susceptibility to immune damage undergone by developing schistosomula involves a change unrelated to the masking of parasite antigens by host molecules. *J. Exp. Med.* 152: 41-53.

- Mota-Santos T.A., C.A.P. Tavares, G. Gazzinelli and J. Pellegrino
1977 Immunosuppression mediated by adult worms in chronic schistosomiasis mansoni. *Am. J. Trop. Med. Hyg.* 26: 727-731.
- Movsesijan M. 1968 Immunoglobulin binding by the miracidia of *Fasciola hepatica*. *J. Comp. Pathol. Ther.* 78: 421-425.
- Movsesijan M. and D. Borojevic 1973 Antigenic analysis of *Fasciola hepatica*: Extraction and fractionation. In: *Isotopes and Radiation in Parasitology*. International Atomic Energy Agency, Vienna. p. 11-22.
- Movsesijan M. and K. Cuperlovic 1973 Pathophysiology and immunology of infections with non-irradiated and irradiated metacercariae of *Fasciola hepatica*. In: *Isotopes and Radiation in Parasitology*. International Atomic Energy Agency, Vienna. p. 23-34.
- Movsesijan M., B. Jovanovic, A. Aalund and P. Nansen 1975 Immune response of sheep to *Fasciola hepatica* infection. *Res. Vet. Sci.* 18: 171-174.
- Murray M. and B. Rushton 1975 The pathology of fascioliasis with particular reference to hepatic fibrosis. In: A.E.R. Taylor and R. Muller *Pathogenic Processes in Parasitic Infections*. Blackwell Scientific Publications, Oxford, Vol. 13, p. 27-41.
- Murrell K.D. and B. Clay 1972 *In vitro* detection of cytotoxic antibodies to *Schistosoma mansoni* schistosomules. *Am. J. Trop. Med. Hyg.* 21: 569-577.
- Myles D.G., P. Primakoff and A.R. Bellve 1981 Surface domains of guinea pig sperm defined with monoclonal antibodies. *Cell* 23: 433-440.

- Nabholz M., M. Cianfriglia, O. Acuto, A. Conzelmann, W. Hass,
H. Von Boehmer, H.R. McDonald, H. Pohlit and J.P. Johnson
1980 Cytolytically active T cell hybrids. *Nature* 287: 437-440.
- Nansen P. 1975 Resistance in cattle to *Fasciola hepatica* induced
by γ ray attenuated larvae: Results from a controlled field trial.
Res. Vet. Sci. 19: 278-283.
- Nansen P., S. Anderson, E. Harmer and H.J. Riising 1972 Experimental
fascioliasis in the pig. *Expl. Parasitol.* 31: 247-254.
- Nash T.E., B. Prescott and F.A. Neva 1974 The characteristics of a
circulating antigen in schistosomiasis. *J. Immunol.* 112: 1500-1507.
- Ng A.K., F. Indiveri, M.A. Pellegrino, G.A. Molinaro, V. Quaranta and
S. Ferrone 1980 Natural cytotoxicity and antibody-dependent
cellular cytotoxicity of human lymphocytes depleted of HLA-DR
bearing cells with monoclonal HLA-DR antibodies. *J. Immunol.* 124:
2336-2340.
- Nossal G.J.V. 1978 Antibodies and Immunity. Second Edition, Basic
Books, New York.
- Notenboom R.H., C-T. Chou, P.W. Good, S. Dubiski, B. Cinader and
G. Köhler 1980 Isolation and characterisation of a mouse-rabbit
hybridoma. *J. Immunogenet.* 7: 359-368.
- O'Beirne A.J. and H.R. Cooper 1979 Heterogenous enzyme immunoassay.
J. Histochem. Cytochem. 27: 1148-1162.
- O'Donnell I.J. and G.F. Mitchell 1980 An investigation of the
antigens of *Ascaris lumbricoides* using a radioimmunoassay and sera
of naturally infected humans. *Int. Arch. Aller. App. Immunol.* 61:
213-219.

- Oellerich M. 1980 Enzyme immunoassays in clinical chemistry: Present status and trends. *J. Clin. Chem. Clin. Biochem.* 18: 197-208.
- Ogilvie B.M. and M.E. Rose 1978 The response of the host to some parasites of the small intestine: Coccidia and nematodes. *INSERM* 72: 237-248.
- Oguz T., H. Grelck, R. Tinar, A. Burgu and M. Alabay 1978 Deneysel olarak enfekte edilen koyunlarda *Fasciola hepatica* 'nın immunoperoksidaz ve immunofloresan teknikleri ile mukayeseli teshisleri. *Ankara Uni. Vet. Fak. Dergisi* 25: 537-553.
- Oi V.T. and L.A. Herzenberg 1980 Immunoglobulin-producing hybrid cell lines. In: B.B. Mishell and S.M. Shiigi *Selected Methods in Cellular Immunology* Freeman, San Francisco. p. 351-373.
- Oi V.T., P.P. Jones, J.W. Goding, L.A. Herzenberg and L.A. Herzenberg 1978 Properties of monoclonal antibodies to mouse Ig allotypes, H-2 and Ia antigens. *Curr. Top. Microbiol. Immunol.* 81: 115-129.
- Olsson L. and H.S. Kaplan 1980 Human-human hybridomas producing monoclonal antibodies of predefined antigenic specificity. *Proc. Natl. Acad. Sci.* 77: 5429-5431.
- Onawunmi O.A. and G.C. Coles 1980 Attempts to immunise sheep with culture antigens of *Taenia hydatigena*. *Res. Vet. Sci.* 29: 122-123.
- Ottesen E.A. and R.W. Poindexter 1980 Modulation of the host response in human schistosomiasis. II. Humoral factors which inhibit lymphocyte proliferative responses to parasite antigens. *Am. J. Trop. Med. Hyg.* 29: 592-597.

- Ouassi M.A., F. Santoro and A. Capron 1980 Interaction between *Schistosoma mansoni* and the complement system. Receptors for C3b on cercariae and schistosomula. *Immunol. Lett.* 1: 197-201.
- Pages J.M. and A.E. Bussard 1978 Establishment and characterization of a perminant murine hybridoma secreting monoclonal autoantibodies. *Cell. Immunol.* 41: 188-194.
- Pain D. and A. Surolia 1979 Protein A-enzyme monoconjugate as versatile tool for enzyme immunoassays. *Febs Letters* 107: 73-76.
- Pantelouris E.M. 1965 The Common Liver Fluke. Permagon Press, Oxford.
- Pappas P.W. and C.P. Read 1975 Membrane transport in helminth parasites: A review. *Expl. Parasitol.* 37: 469-530.
- Parham P. 1979 Purification of immunologically active HLA-A and -B antigens by a series of monoclonal antibody columns. *J. Biol. Chem.* 254: 8709-8712.
- Parish C.R. and J.A. Haywood 1974 The lymphocyte surface. I. Relation between Fc receptors, C'3 receptors and surface immunoglobulin. *Proc. R. Soc. Lond. B.* 187: 47-63.
- Parish C.R. and I.F.C. McKenzie 1978 A sensitive rosetting method for detecting subpopulations of lymphocytes which react with alloantisera. *J. Immunol. Methods* 20: 173-183.
- Parks D.R., V.M. Bryan, V.T. Oi and L.A. Herzenberg 1979 Antigen-specific identification and cloning of hybridomas with a fluorescence-activated cell sorter. *Proc. Natl. Acad. Sci.* 76: 1962-1966.

- Pearson T., G. Galfre, A. Ziegler and C. Milstein 1977 A myeloma hybrid producing antibody specific for an allotypic determinant on "IgD-like" molecules of the mouse. *Eur. J. Immunol.* 7: 684-689.
- Pearson T.W. and N.L. Anderson 1980 Analytical techniques for cell fractions. XXVIII. Dissection of complex antigenic mixtures using monoclonal antibodies and two-dimensional gel electrophoresis. *Anal. Bioc.* 101: 377-386.
- Pearson T.W., M. Pinder, G.E. Roelants, S.K. Kar, L.B. Lundin, K.S. Mayor-Wither and R.S. Hewett 1980 Methods for derivation and detection of anti-parasite monoclonal antibodies. *J. Immunol. Methods* 34: 141-154.
- Pearson T.W., S.K. Kar, T.C. McGuire and L.B. Lundin 1981 Trypanosome variable surface antigens: Studies using two-dimensional gel electrophoresis and monoclonal antibodies. *J. Immunol.* 126: 823-828.
- Peiris J.S.M., S. Gordon, J.C. Unkeless and J.S. Porterfield 1981 Monoclonal anti-Fc receptor IgG blocks antibody enhancement of viral replication in macrophages. *Nature* 289: 189-191.
- Pelley R.P. and G.V. Hillyer 1978 Demonstration of a common antigen between *Schistosoma mansoni* and *Fasciola hepatica*. *Am. J. Trop. Med. Hyg.* 27: 1192-1194.
- Pelley R.P., J.J. Ruffier and K.S. Warren 1976 Suppressive effect of a chronic helminth infection, schistosomiasis mansoni, on the *in vitro* responses of spleen and lymph node cells to the T cell mitogens phytohemagglutinin and concanavalin A. *Infect. Immun.* 13: 1176-1183.

- Pelley R.P., K.S. Warren and P. Jordan 1977 Purified antigen radioimmunoassay in serological diagnosis of schistosomiasis mansoni. *Lancet* 2, 781-785.
- Perez H.A. and S.R. Smithers 1977 *Schistosoma mansoni* in the rat: The adherence of macrophages to schistosomula *in vitro* after sensitization with immune serum. *Int. J. Parasitol.* 7: 315-320.
- Perricone R., L. Fontana, C. De Carolis and P. Ottaviani 1980 Activation of alternative complement pathway by fluid from hydatid cysts. *New Eng. J. Med.* 302: 808-809.
- Perrin L.H., E. Ramirez, L. Er-Hsiang and P.H. Lambert 1980 *Plasmodium falciparum*: Characterization of defined antigens by monoclonal antibodies. *Clin. Exp. Immunol.* 41: 91-96.
- Perrin L.H., E. Ramirez, P.H. Lambert and P.A. Miescher 1981 Inhibition of *Plasmodium falciparum* growth in human erythrocytes by monoclonal antibodies. *Nature* 289: 301-303.
- Petrovic M. and A.M. Deelder 1979 Examination of the possibility of using the defined antigen substrate spheres (DASS) system for immunodiagnosis of *Fasciola hepatica* infection. *Periodi. Biolog.* 81: 513-515.
- Philipp M., R.M.E. Parkhouse and B.M. Ogilvie 1980 Changing proteins on the surface of a parasitic nematode. *Nature* 287: 538-540.
- Phillips D.J., A.P. Kendal, R.G. Webster, P.M. Feorino and C.B. Reimer 1980 Detection of monoclonal influenza antibodies synthesized in culture by hybridoma cells with a solid phase indirect immunofluorometric assay. *J. Immunol. Methods* 1: 275-284.

- Phillips S.M. and D.G. Colley 1978 Immunologic aspects of host responses to Schistosomiasis: Resistance, immunopathology and eosinophil involvement. *Prog. Allergy* 24: 49-182.
- Pierres M., S-T. Ju, C. Waltenbaugh, M.E. Dorf, B. Bencerraf and R.N. Germain 1979 Fine specificity of antibodies to poly (Glu⁶⁰Ala³⁰Tyr¹⁰) produced by hybrid cell lines. *Proc. Natl. Acad. Sci.* 76: 2425-2429.
- Pinder M. and R.S. Hewett 1980 Monoclonal antibodies detect antigenic diversity in *Theileria parva* parasites. *J. Immunol.* 124: 1000-1001.
- Pinder M., A.J. Musoke, W.I. Morrison and G.E. Roelants 1980 The bovine lymphoid system. III. A monoclonal antibody specific for bovine cell surface and serum IgM. *Immunology* 40: 359-365.
- Poluektova P.F. 1978 Serum immunoglobulins in animals infected with *Fasciola hepatica* (Communication 1). Quantitative analysis of IgM and IgG classes in the sera of rabbits and guinea pigs with experimental fascioliasis. *Trudy Vses. Instit. Gel'mintol. Skyrabina* 24: 99-105.
- Pontecorvo G., P.N. Riddle and A. Hales 1977 Time and mode of fusion of human fibroblasts treated with polyethylene glycol (PEG). *Nature* 265: 257-258.
- Poste G. and G. Nicolson 1978 Membrane fusion. *Cell Surface Rev.* 5, North-Holland, Amsterdam.

Potocnjak P., N. Yoshida, R.S. Nussenzweig and V. Nussenzweig 1980

Monovalent fragments (Fab) of monoclonal antibodies to a sporozoite surface antigen (Pb44) protect mice against malarial infection. *J. Exp. Med.* 151: 1504-1513.

Potter M. 1972 Immunoglobulin-producing tumours and myeloma proteins of mice. *Physiol Rev.* 52: 631-719.

Prichard R.K., C.A. Hall, J.D. Kelly, I.C.A. Martin and A.D. Donald

1980 The problem of anthelmintic resistance in nematodes. *Aust. Vet. J.* 56: 239-251.

Rajasekariah G.R. 1977 Immunity to *Fasciola hepatica* in rats.

Ph.D. Thesis, Australian National University.

Rajasekariah G.R. and M.J. Howell 1977a The fate of *Fasciola hepatica*

metacercariae following challenge infection in immune rats.

J. Helminthol. 51: 289-294.

Rajasekariah G.R. and M.J. Howell 1977b *Fasciola hepatica* in rats:

Effects of host age and infective dose. *Int. J. Parasitol.* 7:

119-121.

Rajasekariah G.R. and M.J. Howell 1978a *Fasciola hepatica*: Role of

developmental stages in the rat's resistance to challenge. *Expl.*

Parasitol. 44: 233-238.

Rajasekariah G.R. and M.J. Howell 1978b Acquired immunity to the

trematode *Fasciola hepatica* in rats. *Aust. J. Exp. Biol. Med.*

Sci. 56: 747-756.

Rajasekariah G.R. and M.J. Howell 1979 *Fasciola hepatica* in rats:

Transfer of immunity by serum and cells from infected to *F. hepatica*

naive animals. *J. Parasitol.* 65: 481-487.

- Rajasekariah G.R. and M.J. Howell 1980 Enzyme assay for detecting resistance to reinfection with the liver fluke *Fasciola hepatica* in rats. *Res. Vet. Sci.* 29: 124-125.
- Rajasekariah G.R. and M.J. Howell 1981 Age-associated responses in susceptible and resistant rats to infection with *Fasciola hepatica*. *Int. J. Parasitol.* 11: 59-65.
- Rajasekariah G.R., M.D. Rickard, P.E. Montague and G.F. Mitchell 1979a Attempts to immunise rats and mice against infection with *Fasciola hepatica* using antigens prepared from *Taenia hydatigena*. *Z. Parasitenkd.* 58: 175-180.
- Rajasekariah G.R., G.F. Mitchell, C.B. Chapman and P.E. Montague 1979b *Fasciola hepatica*: Attempts to induce protection against infection in rats and mice by injection of excretory/secretory products of immature worms. *Parasitology* 79: 393-400.
- Rajasekariah G.R., M.D. Rickard and G.F. Mitchell 1980 Immunization of mice against infection with *Taenia taeniaeformis* using various antigens prepared from eggs, oncospheres, developing larvae and strobilocerci. *Int. J. Parasitol.* 10: 315-324.
- Ramalho-Pinto F.J., D.J. McLaren and S.R. Smithers 1978 Complement mediated killing of schistosomula of *Schistosoma mansoni* by rat eosinophils *in vitro*. *J. Exp. Med.* 147: 147-156.
- Ramalho-Pinto F.J., R. De Rossi and S.R. Smithers 1970 Murine *Schistosomiasis mansoni*: Anti-schistosomula antibodies and the IgG subclasses involved in the complement- and eosinophil-mediated killing of schistosomula *in vitro*. *Parasite Immunol.* 1: 295-308.

- Ramshaw I.A. and C.R. Parish 1977 Enhanced antibody responses to antigens presented on autologous erythrocytes. *J. Immunol. Methods* 18: 251-255.
- Raschke W.C. 1978 Expression of murine IgM, IgD and Ia molecules on hybrids of murine LPS blasts with a Syrian hamster B lymphoma. *Curr. Top. Microbiol. Immunol.* 81: 70-76.
- Raschke W.C. 1980 Plasmacytomas, lymphomas and hybridomas: Their contribution to immunology and molecular biology. *Biochim. Biophys. Acta* 605: 113-145.
- Rathjen D., M. Gorrell and J. Chin 1981 Screening procedures for detecting monoclonal antibodies to protoplast membrane antigens. *Proc. Aust. Bioc. Soc.* 14: 109.
- Rau M.E. and C.E. Tanner 1975 BCG suppresses growth and metastasis of hydatid infections. *Nature* 256: 318-319.
- Raulet D.H., P.D. Gottlieb and M.J. Bevan 1980 Fractionation of lymphocyte populations with monoclonal antibodies specific for Lyt 2.2 and Lyt 3.1. *J. Immunol.* 125: 1136-1143.
- Reinherz E.L., P.C. Kung, G. Goldstein and S.F. Schlossman 1979 A monoclonal antibody with selective reactivity with functionally mature human thymocytes and all peripheral human T cells. *J. Immunol.* 123: 1312-1317.
- Reinherz E.L., L. Moretta, M. Roper, J.M. Breard, M.C. Mingari, M.D. Cooper and S.F. Schlossman 1980 Human T lymphocyte subpopulations defined by Fc receptors and monoclonal antibodies. *J. Exp. Med.* 151: 969-974.

- Rembaum A. and W.J. Dreyer 1980 Immunomicrospheres: Reagents for cell labelling and separation. *Science* 208: 364-368.
- Rener J., R. Carter, Y. Rosenberg and L.H. Miller 1980 Anti-gamete monoclonal antibodies synergistically block transmission of malaria by preventing fertilization in the mosquito. *Proc. Natl. Acad. Sci.* 77: 6797-6799.
- Reuben J.M., C.E. Tanner and M.E. Rau 1978 Immunoprophylaxis with BCG of experimental *Echinococcus multilocularis* infections. *Infect. Immun.* 21: 135-139.
- Rickard M.D. and K.J. Bell 1971 Successful vaccination of lambs against infection with *Taenia ovis* using antigens produced during *in vitro* cultivation of larval stages. *Res. Vet. Sci.* 12: 401-402.
- Rickard M.D. and A.J. Adolph 1977 Vaccination of lambs against infection with *Taenia ovis* using antigens collected during short-term *in vitro* incubation of activated *T. ovis* oncospheres. *Parasitology* 75: 183-188.
- Rifkin G.G. and C. Dobson 1979 Predicting resistance of sheep to *Haemonchus contortus* infections. *Vet. Parasitol.* 5: 365-378.
- Ringertz N.R. and R.E. Savage 1976 Cell Hybrids. Academic Press, N.Y.
- Robert R., P. Leynia de la Jarrige, D. Chabasse, C. Mahaza, C. Bizon and H. Genthon 1980 Contribution au diagnostic immunologique de la fasciolose à *F. hepatica* chez les bovins: Recherche d'Ag fraction II et d'Ac anti-fraction II. *Rec. Méd. Vét.* 156: 533-538.

- Romagnani S., G.F. Del Prete, G.M. Giudizi, F. Almeriogogna and M. Ricci 1979 A simple solid-phase radioimmunoassay for the measurement of IgG secreted *in vitro* by human lymphocytes. *J. Immunol. Methods* 29: 263-270.
- Rose M.E., B.M. Ogilvie, P. Hesketh and M.F.W. Festing 1979 Failure of nude (athymic) rats to become resistant to reinfection with the intestinal coccidian parasite *Eimeria nieschulzi* or the nematode *Nippostrongylus brasiliensis*. *Parasite Immunol.* 1: 125-132.
- Ross J.G. 1965 Experimental infections of cattle with *Fasciola hepatica*, a comparison of low and high infection rates. *Nature* 208: 907.
- Ross J.G. 1966a Studies on immunity to *Fasciola hepatica*. Naturally acquired immunity in rabbits. *Br. Vet. J.* 122: 209-211.
- Ross J.G. 1966b Experimental infections of cattle with *Fasciola hepatica*. Challenge infections. *Nature* 212: 1464-1465.
- Ross J.G. 1967a Experimental infections of cattle with *Fasciola hepatica*: High level single infections in calves. *J. Helminthol.* 41: 217-222.
- Ross J.G. 1967b Experimental infections of cattle with *Fasciola hepatica*: The production of an acquired self-cure by challenge infection. *J. Helminthol.* 41: 223-228.
- Ross J.G. 1967c Studies of immunity to *Fasciola hepatica*. Acquired immunity in cattle, sheep and rabbits following natural infection and vaccine procedures. *J. Helminthol.* 41: 393-399.

- Ross J.G., J.R. Todd and C. Dow 1966 Single experimental infections of calves with the liver fluke, *Fasciola hepatica* (Linnaeus 1758). *J. Comp. Pathol.* 76: 67-81.
- Ross J.G., C. Dow and J.R. Todd 1967 The pathology of *Fasciola hepatica* infections in pigs: A comparison of the infections in pigs and other hosts. *Br. Vet. J.* 123: 317-321.
- Ross M.E., D.J. Reis and T.H. Joh 1981 Monoclonal antibodies to tyrosine hydroxylase: Production and characterization. *Brain Res.* 208: 493-498.
- Rotmans J.P. and G.W. Mooij 1980 Separation and comparative immunoassay (DASS, ELISA) with antigens from adult *Schistosoma mansoni*. *Trans. R. Soc. Trop. Med. Hyg.* 74: 463-468.
- Rousseaux J., M.T. Picque, H. Bazin and G. Biserte 1981 Rat IgG subclasses: Differences in affinity to protein A-sepharose. *Mol. Immunol.* 18: 639-645.
- Rowlands D. ap T. and R.B. Clampitt 1979 Plasma enzyme levels in ruminants infected with *Fasciola hepatica*. *Vet. Parasitol.* 5: 155-175.
- Rubin R.L., M.A. Hardtke and R.I. Carr 1980 The effect of high antigen density on solid phase radioimmunoassays for antibody regardless of immunoglobulin class. *J. Immunol. Methods* 33: 277-292.
- Ruitenbergh E.J., P.A. Steerenberg, B.J.M. Brosi and J. Buys 1974 Serodiagnosis of *Trichinella spiralis* infections in pigs by enzyme-linked immunosorbent assays. *Bull. WHO* 51: 108-109.

- Rushton B. 1977 Ovine fascioliasis following reinfection. *Res. Vet. Sci.* 22: 133-34.
- Said S.B. and M. Handlos 1980 Untersuchungen einiger blutparameter an schafen mit einer provozierten, natürlichen infestation von *Fasciola hepatica*. *Prak. Tier.* 61: 489-498.
- Salonen E. A-M. and A. Vaheri 1979 Immobilization of viral and mycoplasma antigens and of immunoglobulins on polystyrene surface for immunoassays. *J. Immunol. Methods* 30: 209-218.
- Samuelson J.C., A. Sher and J.P. Caulfield 1980 Newly transformed schistosomula spontaneously lose surface antigens and C3 acceptor sites during culture. *J. Immunol.* 124: 2055-2057.
- Sandeman R.M. 1980 The immune response of sheep to infection with *Fasciola hepatica*. Ph.D. Thesis, Australian National University.
- Sandeman R.M. and M.J. Howell 1980 *In vitro* studies of the response of sheep to infection with *Fasciola hepatica*. *Vet. Parasitol.* 6: 347-357.
- Sandeman R.M. and M.J. Howell 1981a Precipitating antibodies against excretory/secretory antigens of *Fasciola hepatica*. *Vet. Parasitol.* In press.
- Sandeman R.M. and M.J. Howell 1981b Response of sheep to challenge infection with *Fasciola hepatica*. *Res. Vet. Sci.* 30: 294-297.
- Sandeman R.M. and M.J. Howell 1981c Characterisation of sheep antibodies in precipitate formed *in vitro* with surface antigens of *Fasciola hepatica*. In preparation.

- Sandeman R.M., M.J. Howell and N.J. Campbell 1980 An attempt to vaccinate sheep against *Fasciola hepatica* using a juvenile fluke antigen sheep antibody complex. *Res. Vet. Sci.* 29: 255-259.
- Sanderson C.J., A.F. Lopez and M.M. Bunn Moreno 1977 Eosinophils and not lymphoid K cells kill *Trypanosoma cruzi* epimastigotes. *Nature* 268: 340-341.
- Santoro F., Y. Carlier, R. Borojevic, D. Bout, P. Tachon and A. Capron 1977 Parasitic antigen in milk from mothers infected with *Schistosoma mansoni*. *Ann. Trop. Med. Parasitol.* 71: 121-123.
- Santoro F., J. Bernal and A. Capron 1979 Complement activation by parasites. *Acta Trop.* 36: 5-14.
- Santoro F., B. Vandemeulebroucke and A. Capron 1979 *Schistosoma mansoni*. Circulating antigens and immune complexes in infected mice. *Expl. Parasitol.* 47: 392-402.
- Santoro F., M.A. Ouaisi, J. Pestel and A. Capron 1980a Interaction between *Schistosoma mansoni* and the complement system: Binding of Clq to schistosomula. *J. Immunol.* 124: 2886-2891.
- Santoro F., J. Pestel, T. Le Presle, M.C. Liebart and A. Capron 1980b *Schistosoma mansoni* anticomplementary antigens (SACA). Detection in schistosomula and adult worms. *Immunol. Lett.* 2: 43-46.
- Saunders G.C. 1979 The art of solid-phase enzyme immunoassay including selected protocols: In: R.M. Nakamura, W.R. Dito and E.S. Tucker III. *Immunoassays in the Clinical Laboratory*. Alan R. Liss Inc., N.Y., p. 99-118.

- Saxena R.K., W.H. Adler and A.A. Nordin 1980 Monocolonal anti-Thy 1.2. antibodies from hybridomas H013-4 do not react with mouse natural killer cells. *Immunol. Comm.* 9: 371-378.
- Scharff M.D. 1979 Cell hybrids in immunology. *Fed. Proc.* 38: 2442-2443.
- Schinski V.D., W.C. Clutter and K.D. Murrell 1976 Enzyme and ^{125}I -labelled anti-immunoglobulin assays in the immunodiagnosis of schistosomiasis. *Am. J. Trop. Med. Hyg.* 25: 824-831.
- Schneider M.D. and G.S. Eisenbarth 1979 Transfer plate radioassay using cell monolayers to detect anti-cell surface antibodies synthesized by lymphocyte hybridomas. *J. Immunol. Methods* 29: 331-342.
- Schrader J.W. and I. Clark-Lewis 1981 T cell hybridoma-derived regulatory factors. I. Production of T cell growth factor following stimulation by concanavalin A. *J. Immunol.* 126: 1101-1105.
- Schroder J. 1980 Monoclonal antibodies: A new tool for research and immunodiagnostics. *Med. Biol. (Helsinki)* 58: 140-148.
- Schulman M., C.D. Wilde and G. Köhler 1978 A better cell line for making hybridomas secreting specific antibodies. *Nature* 276: 269-270.
- Schulman M. and G. Köhler 1979 Fusion of immunoglobulin secreting cells. In: B. Pernis and H. Vogel *Cells of Immunoglobulin Synthesis*. Academic Press, N.Y., p. 275-293.
- Schwader J. and E.P. Cohen 1973 Human x mouse somatic cell hybrid clone secreting immunoglobulins of both parental types. *Nature* 224: 444-447.

- Secher D.S. and D.C. Burke 1980 A monoclonal antibody for large-scale purification of human leukocyte interferon. *Nature* 285: 446-450.
- Seravalli E., R. Schwab, B. Pernis and M. Siniscalco 1978 Characterization of a mouse myeloma lymphoblast hybrid line with respect to Ig production and species-specific surface antigens. *Cytogenet. Cell Genet.* 22: 260-264.
- Sethi K.K., T. Endo and H. Brandis 1980 Hybridomas secreting monoclonal antibody with specificity for *Toxoplasma gondii*. *J. Parasitol.* 66: 192-196.
- Sewell M.M.H. 1964 The immunology of fascioliasis II. Quantitative studies on the precipitin reaction. *Immunology* 7: 671-680.
- Sharon J., S.L. Morrison and E.A. Kabat 1979 Detection of specific hybridoma clones by replica immunoadsorption of their secreted antibodies. *Proc. Natl. Acad. Sci.* 76: 1420-1424.
- Sher A. 1976 Complement-dependent adherence of mast cells to schistosomula. *Nature* 263: 334-336.
- Sher A. and S. McIntyre 1977 Receptors for C3 on rat peritoneal mast cells. *J. Immunol.* 119: 722-725.
- Sher A., B.F. Hall and M.A. Vadas 1978 Acquisition of murine major histocompatibility complex gene products by schistosomula of *Schistosoma mansoni*. *J. Exp. Med.* 148: 46-57.
- Shigeta M., T. Watanabe, S. Maruyama, K. Koyama and S. Isojima 1980 Sperm immobilizing monoclonal antibody to human seminal plasma antigens. *Clin. Exp. Immunol.* 42: 458-462.

- Shih J.W-K., P.J. Cote, G.M. Dapolita and J.L. Gerin 1980 Production of monoclonal antibodies against hepatitis B surface antigen by somatic cell hybrids. *J. Virol. Methods* 1: 257-274.
- Sigal N.H. and N.R. Klinman 1978 The B-cell clonotype repertoire. *Adv. Immunol.* 26: 255-337.
- Simpkin K.G., C.R. Chapman and G.C. Coles 1980 *Fasciola hepatica*: A proteolytic digestive enzyme. *Expl. Parasitol.* 49: 281-287.
- Sinclair K.B. 1970 The effect of splenectomy on the pathogenicity of *Fasciola hepatica* in sheep. *Br. Vet. J.* 126: 15-29.
- Sinclair K.B. 1971a Acquired resistance to *Fasciola hepatica* in sheep. *Br. Vet. J.* 127: 125-136.
- Sinclair K.B. 1971b Resistance to *Fasciola hepatica* in sheep. Attempts to transfer resistance with lymph node and spleen homogenates. *Br. Vet. J.* 127: 408-419.
- Sinclair K.B. 1973 The resistance of sheep to *Fasciola hepatica*: Studies of the development and pathogenicity of challenge infections. *Br. Vet. J.* 129: 236-250.
- Sinclair I.J. and L.P. Joyner 1974 The effect of administration of a homologous antigen on the establishment of *Fasciola hepatica* in the rabbit. *Res. Vet. Sci.* 16: 320-327.
- Sinclair I.J. and S.B. Kendall 1969 Precipitating antibodies to *Fasciola hepatica* in rabbits. *Res. Vet. Sci.* 10: 483-485.
- Sinclair K.B. 1975 The resistance of sheep to *Fasciola hepatica*. Studies on the pathophysiology of challenge infections. *Res. Vet. Sci.* 19: 296-303.

- Sirag S.B., N.O. Christensen, P. Nansen, J. Monrad and F. Frandsen
1981 Resistance to *Fasciola hepatica* in calves harbouring primary patent *Schistosoma bovis* infections. *J. Helminthol.* 55: 63-70.
- Smith M. and K. Hirschhorn 1978 Studies on the synthesis of immunoglobulins by man-mouse somatic cell hybrids. *Cytogenet. Cell Genet.* 22: 275-280.
- Smith M. and G. Webbe 1974 Damage to schistosomula of *Schistosoma haematobium* *in vitro* by immune baboon and human sera and absence of cross-reaction with *Schistosoma mansoni*. *Trans. R. Soc. Trop. Med. Hyg.* 68: 70.
- Smithers S.R. 1976 Immunity to trematode infections with special reference to schistosomiasis and fascioliasis. In: S. Cohen and E.H. Sadun *Immunology of Parasitic Infections*. Blackwell Scientific, Oxford, p. 296-332.
- Smithers S.R., R.J. Terry and D.J. Hockley 1969 Host antigens in schistosomiasis. *Proc. R. Soc. Ser. B.* 171: 483-494.
- Snary D., M.A. Smith and J.A. Clegg 1980 Surface proteins of *Schistosoma mansoni* and their expression during morphogenesis. *Eur. J. Immunol.* 10: 573-575.
- Sogandares-Bernal F., M.C. Race, M.V. Dennis and M. Voge 1981 Circulating antigens in infections of mice by tetrathyridia of *Mesocestoides corti* Hoeppli, 1925. *Z. Parasitkd.* 64: 157-167.
- Solomon G.B. 1969 Host hormones and parasitic infection. *Int. Rev. Trop. Med.* 3: 101-158.

- Solter D. and B.B. Knowles 1978 Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1). *Proc. Natl. Acad. Sci.* 75: 5565-5569.
- Soulsby E.J.L. 1957 An antagonistic action of sheep serum on the miracidia of *Fasciola hepatica*. *J. Helminthol.* 31: 161-170.
- Soulsby E.J.L. 1979 The immune system and helminth infection in domestic species. *Adv. Vet.Sci. Comp. Med.* 23: 71-102.
- Springer T.A. 1980 Quantitation of light chain synthesis in myeloma x spleen cell hybrids and identification of myeloma chain loss variants using radioimmunoassay. *J. Immunol. Methods* 37: 139-152.
- Springer T.A., G. Galfre, D.S. Secher and C. Milstein 1978 Monoclonal xenogeneic antibodies to murine cell surface antigens: Identification of novel leukocyte differentiation antigens. *Eur. J. Immunol.* 8: 539-551.
- Srendi B., H.Y. Tse, C. Chen and R.H. Schwartz 1981 Antigen-specific clones of proliferating T lymphocytes. I. Methodology, specificity and MHC restriction. *J. Immunol.* 126: 341-347.
- Stähli C., T. Staehelin, V. Miggiano, J. Schmidt and P. Häring 1980 High frequencies of antigen-specific hybridomas: Dependence on immunization parameters and prediction by spleen cell analysis. *J. Immunol. Methods* 32: 297-304.
- Staines N.A. and A.M. Lew 1980 Whither monoclonal antibodies? *Immunology* 40: 287-293.
- Starling J.J., C.R. Klein and K.D. Noonan 1979 Production of monoclonal antibodies against a cell surface concanavalin A binding glycoprotein. *J. Supramol. Struct.* 11: 563-577.

Steinitz M., G. Klein, S. Koskimies and O. Makela 1977 EB virus - induced B lymphocyte cell lines producing specific antibody.

Nature 269: 420-422.

Steplewski Z., M. Herlyn, D. Herlyn, W.H. Clark and H. Koprowski 1979 Reactivity of monoclonal anti-melanoma antibodies with melanoma cells freshly isolated from primary and metastatic melanoma. *Eur. J. Immunol.* 9: 94-96.

Stern P.L., K.R. Willison, E. Lennox, G. Galfre, C. Milstein, D. Secher, A. Ziegler and T. Springer 1978 Monoclonal antibodies as probes for differentiation and tumour-associated antigens: A Forssman specificity on tetratocarcinoma stem cells. *Cell* 14: 775-783.

Sunderland C.A., W.R. McMaster and A.F. Williams 1979 Purification with monoclonal antibody of a predominant leukocyte-common antigen and glycoprotein from rat thymocytes. *Eur J. Immunol.* 9: 155-159.

Sykes A.R., R.L. Coop and B. Rushton 1980a Chronic subclinical fascioliasis in sheep: Effects of food intake, food utilisation and blood constituents. *Res. Vet. Sci.* 28: 63-70.

Sykes A.R., R.L. Coop and M.G. Robinson 1980b Chronic subclinical ovine fascioliasis: Plasma glutamate dehydrogenase, gamma-glutamyl transpeptidase and aspartate aminotransferase activities and their significance as diagnostic aids. *Res. Vet. Sci.* 28: 71-75.

Tailliez R. and S. Korach 1970a Les antigènes de *Fasciola hepatica*.

I. Isolement et caractérisation d'un antigène spécifique du genre.

Ann. Inst. Past. Paris 118: 61-78.

Tailliez R. and S. Korach 1970b Les antigènes de *Fasciola hepatica*.

II. Étude immunologique et localisation *in situ* d'un antigène spécifique du genre. *Ann. Inst. Past. Paris* 118: 330-339.

Tailliez R., R. Mangalo and S. Korach 1967 Isolement d'un antigène spécifique de la grand douve du foie (*Fasciola hepatica* L.). *C.R. Acad. Sci. Ser. D* 265: 466-469.

Tanaka H., H. Matsuda and J.S. Nosenas 1979 Detection of antibodies in *Schistosoma japonicum* infections by a microtechnique of enzyme-linked immunosorbent assay (ELISA). *Jap. J. Exp. Med.* 49: 289-292.

Tanaka T., K. Takaya, T. Kunimoto and H. Baba 1979 Effects of latex particles on immune responses of the murine host and on macrophages *in vitro*. *Int. J. Immunopharm.* 1: 197-203.

Tarleton R.L. and W.M. Kemp 1981 Demonstration of IgG-Fc and C3 receptors on adult *Schistosoma mansoni*. *J. Immunol.* 126: 379-384.

Tavares C.A.P., G. Gazzinelli, T.A. Mota-Santos and W. Dias Da Silva 1978 *Schistosoma mansoni*: Complement-mediated cytotoxic activity *in vitro* and effect of de complementation on acquired immunity in mice. *Expl. Parasitol.* 46: 145-151.

Tavares C.A.P., M.N. Cordiero, T.A. Mota-Santos and G. Gazzinelli 1980 Artificially transformed schistosomula of *Schistosoma mansoni*: Mechanism of acquisition of protection against antibody-mediated killing. *Parasitology* 80: 95-104.

Taylor M.G., E.R. James, G.S. Nelson, Q. Bickle, D.W. Dunne and G. Webbe 1976 Immunization of sheep against *Schistosoma mattheei* using either irradiated cercariae or irradiated schistosomula. *J. Helminthol.* 50: 1-9.

- Taylor M.G., E.R. James, Q.D. Bickle, M.J. Doenhoff and G.S. Nelson
1978 Vaccination in schistosomiasis. *INSERM* 72: 291-306.
- Taylor P.M., C.D. Mackenzie and B.M. Ogilvie 1979 *In vitro* cell
mediated death of nematode infective larvae. *Parasitology* 79:
xxxviii.
- Terry R.J. 1978 Immunodepression in parasite infections. *INSERM* 72:
161-178.
- Theofilopoulos A.N. and F.J. Dixon 1979 The biology and detection of
immune complexes. *Adv. Immunol.* 28: 89-221.
- Thompson R.C.A. and M.J. Howell 1979 Effect of BCG on the resistance
of rats to infection with *Fasciola hepatica*. *Z. Parasitenkd.* 61:
93-98.
- Thompson R.C.A. and W.J. Penhale 1978 The enhancement of
tetrathyridial proliferation of *Mesocostoides corti* in mice by
BCG. *Z. Parasitenkd.* 56: 195-203.
- Thorpe E. 1965a The pathology of experimental fascioliasis in the
albino rat. *J. Comp. Pathol.* 75: 39-48.
- Thorpe E. 1965b An immunocytochemical study with *Fasciola hepatica*.
Parasitology 55: 209-214.
- Thorpe E. 1965c Liver damage and the host-parasite relationship in
experimental fascioliasis in the albino rat. *Res. Vet. Sci.* 6:
498-509.
- Thorpe E. and A.W.J. Broome 1962 Immunity to *Fasciola hepatica*
infection in albino rats vaccinated with irradiated metacercariae.
Vet. Rec. 74: 755-756.

- Thorpe E. and E.J.H. Ford 1969 Serum enzyme and hepatic changes in sheep infected with *Fasciola hepatica*. *J. Pathol.* 97: 619-629.
- Thorsell W. and N. Björkman 1965 Morphological and biochemical studies on absorption and secretion in the alimentary tract of *Fasciola hepatica* L. *J. Parasitol.* 51: 217-223.
- Threadgold L.T. 1976 *Fasciola hepatica*: Ultrastructure and histochemistry of the glycocalyx of the tegument. *Expl. Parasitol.* 39: 119-134.
- Threadgold L.T. and G. Brennan 1978 *Fasciola hepatica*: Basal infolds and associated vacuoles of the tegument. *Expl. Parasitol.* 46: 300-316.
- Torpier G., A. Capron and M.A. Ouaisi 1979 Receptor for IgG (Fc) and human β_2 -microglobulin on *S. mansoni* schistosomula. *Nature* 278: 447-449.
- Tribouley J., J. Tribouley-Duet and M. Appriou 1979 Influence d'une réinfestation par le Bacille de Calmette et Guérin (BCG) sur la résistance non spécifique de la souris vis-à-vis de *Schistosoma mansoni*. *Path. Biol.* 27: 491-492.
- Trizio D., C. Della Bruna and A.M. Isetta 1980 Modification of immune responsiveness in murine *Schistosomiasis mansoni*. I. Time course after cercarial exposure. *Immunology* 40: 353-358.
- Trowbridge I.S. 1978 Interspecies spleen-myeloma hybrid producing monoclonal antibodies against mouse lymphocyte surface glycoprotein. *J. Exp. Med.* 148: 313-323.

- Trucco M.M., J.W. Stocker and R. Ceppellini 1978 Monoclonal antibodies against human lymphocyte antigens. *Nature* 273: 666-668.
- Tsu T.T. and L.A. Herzenberg 1980 Solid-phase radioimmune assays. In: B.B. Mishell and S.M. Shiigi 1980 *Selected Methods in Cellular Immunology*. Freeman, San Francisco, p. 373-397.
- Uotila M., E. Engvall and E. Ruoslahti 1980 Monoclonal antibodies to human alphafetoprotein. *Mol. Immunol.* 17: 791-794.
- Uotila M., E. Ruoslahti and E. Engvall 1981 Two-site sandwich enzyme immunoassay with monoclonal antibodies to human alphafetoprotein. *J. Immunol. Methods* 42: 11-15.
- Urquhart G.M. 1980 Application of immunity in the control of parasitic diseases. *Vet. Parasitol.* 6: 217-239.
- Urquhart G.M., W. Mulligan and F.W. Jennings 1954 Artificial immunity to *Fasciola hepatica* in rabbits. *J. Infec. Dis.* 94: 126-133.
- Vadas M.A., J.R. David, A.E. Butterworth, N.T. Pisani and T.A. Siongok 1979 A new method for the purification of human eosinophils and neutrophils and a comparison of the ability of these cells to damage schistosomula of *Schistosoma mansoni*. *J. Immunol.* 122: 1228-1236.
- Van de Zeijst B.A.M., C.C. Stewart and S. Schlesinger 1978 Proliferative capacity of mouse peritoneal macrophages *in vitro*. *J. Exp. Med.* 147: 1253-1266.
- Van Egmond J.G., A.M. Deelder and M.R. Daha 1981 *Schistosoma mansoni*: Complement activation by antigenic preparations. *Expl. Parasitol.* 51: 188-194.

- Van Tiggele L.J. and H.J. Over 1976 Serological diagnosis of fascioliasis. *Vet Parasitol.* 1: 239-248.
- Vernes A., J. Biguet, F. Floch and M.N. Lefebvre 1972 L'hypersensibilité de type retardé au cours des helminthiases expérimentales: Etude du phénomène chez de cobaye *in vivo* et *in vitro*. *Path. Biol.* 20: 24-29.
- Verwaerde C., J.M. Grzych, H. Bazin, M. Capron and A. Capron 1979 Production d'anticorps monoclonaux anti-*Schistosoma mansoni*. Étude préliminaire de leurs activités biologiques. *C.R. Acad. Sci. Paris D* 289: 725-727.
- Voller A., A. Bartlett and D.E. Bidwell 1976a Enzyme immunoassays for parasitic diseases. *Trans. R. Soc. Trop. Med. Hyg.* 70: 98-106.
- Voller A., D.E. Bidwell and A. Bartlett 1976b Enzyme immunoassays in diagnostic medicine. *Bull. W H O* 53: 55-65.
- Voller A., A. Bartlett and D.E. Bidwell 1978 Enzyme immunoassays with special reference to ELISA Techniques. *J. Clin. Pathol.* 31: 507-520.
- Von Lichtenberg F., A. Sher, N. Gibbons and B.L. Doughty 1976 Eosinophil-enriched inflammatory response to schistosomula in the skin of mice immune to *Schistosoma mansoni*. *Amer. J. Pathol.* 84: 479-500.
- Wakelin D. 1978a Genetic control of susceptibility and resistance to parasitic infection. *Adv. Parasitol.* 16: 219-310.
- Wakelin D. 1978b Immunity to intestinal parasites. *Nature* 273: 617-622.

- Wakelin D. and A.M. Donachie 1980 Genetic control of immunity to parasites: Adoptive transfer of immunity between inbred strains of mice characterized by rapid and slow immune expulsion of *Trichinella spiralis*. *Parasite Immunol.* 2: 249-260.
- Walker W.S. 1977 Mediation of macrophage cytolytic and phagocytic activities by antibodies of different classes and class specific Fc-receptors. *J. Immunol.* 119: 367-373.
- Walker A.W. and K.J. Isselbacher 1977 Intestinal antibodies. *N. Eng. J. Med.* 297: 767-773.
- Warren S.L., M. Fanger and K.E. Neet 1980 Inhibition of biological activity of mouse β -nerve growth factor by monoclonal antibody. *Science* 210: 910-912.
- Washington E.A., A.M. Barton, W.L. Nicholas and A.C. Stewart 1982 A comparison between laboratory rats and mice as hosts for the tetrathyridial stage of *Mesocostoides corti*. In preparation.
- Wattre P., M. Capron and A. Capron 1978 Le diagnostic immunologique de la distomatose à *Fasciola hepatica* (à propos de 105 observations). *Lille Méd.* 23: 292-296.
- Wattre P., M. Capron, J-P. Dessaint and A. Capron 1980 Apport de l'immunologie au diagnostic et au traitement des maladies parasitaires. *Rev. Practic.* 30: 969-978.
- Weller P.F. and E.J. Goetzl 1979 The regulatory and effector role of eosinophils. *Adv. Immunol.* 27: 339-371.
- Welsh K.I., G. Dorval and H. Wigzell 1975 Rapid quantitation of membrane antigens. *Nature* 254: 67-69.

Wikerhauser T. 1961 Immunobiological diagnosis of fascioliasis

II. The *in vitro* action of immune serum on the young parasitic stage of *Fasciola hepatica* - a new precipitin test for fascioliasis. *Vet. Archiv. Zagreb* 31: 71-80.

Wilde C.D. and C. Milstein 1980 Analysis of immunoglobulin chain secretion using hybrid myelomas. *Eur. J. Immunol.* 10: 462-467.

Williams J.F. 1979 Recent advances in the immunology of cestode infections. *J. Parasitol.* 65: 337-349.

Wilson R.A. and P.E. Barnes 1977 The formation and turnover of the membranocalyx on the tegument of *Schistosoma mansoni*. *Parasitology* 74: 61-71.

Wilson R.A. and P.E. Barnes 1979 Synthesis of macromolecules by the epithelial surfaces of *Schistosoma mansoni*: An autoradiographic study. *Parasitology* 78: 295-310.

Wilson R.J.M. 1978 Circulating antigens of parasites. *INSERM* 72: 87-102.

World Health Organisation 1977 The role of immune complexes in disease. WHO Technical Report Series No. 606, WHO, Geneva.

World Health Organisation 1979 Parasitic zoonoses. WHO Technical Report Series 637, WHO, Geneva.

Yarmush M.L., F.T. Gates, D.R. Weisfogel and T.J. Kindt 1980 Identification and characterization of rabbit - mouse hybridomas secreting rabbit immunoglobulin chains. *Proc. Natl. Acad. Sci.* 77: 2899-2903.

- Yelton D.E. and M.D. Scharff 1980 Monoclonal antibodies. *Amer. Sci.* 68: 510-516.
- Yelton D.E., B.A. Diamond, S-P. Kwan and M.D. Scharff 1978 Fusion of mouse myeloma and spleen cells. *Curr. Top. Microbiol. Immunol.* 81: 1-7.
- Yolken R.H. 1980 Enzyme-linked immunosorbent assay (ELISA): A practical tool for rapid diagnosis of viruses and other infectious agents. *Yale J. Biol. Med.* 53: 85-92.
- Yoshida N., R.S. Nussenzweig, P. Potocnjak, V. Nussenzweig and M. Aikawa 1980 Hybridoma produces protective antibodies directed against the sporozoite stage of malaria parasite. *Science* 207: 71-73.
- Yoshihara S., K. Suzuki, T. Suto and J. Fujita 1979 Fluorescent antibody studies on the distribution of antigens in *Fasciola* sp. with specific antisera against antigens of agar-gel precipitates. *Jap. J. Vet. Sci.* 41: 227-233.
- Youle R.J. and D.M. Neville 1980 Anti-Thy 1.2 monoclonal antibody linked to ricin is a potent cell-type-specific toxin. *Proc. Natl. Acad. Sci.* 77: 5483-5486.
- Zagury D., L. Phalente, J. Bernard, E. Hollande and G. Buttin 1979 Anti-peroxidase antibody-secreting hybrid lines. I. Identification, cloning and cell characterization. *Eur. J. Immunol.* 9: 1-6.
- Ziegler A. 1980 Monoclonal antibodies as tools in haematology. *Blut* 4: 1-10.

- Zipser B. and R. McKay 1981 Monoclonal antibodies distinguish identifiable neurones in the leech. *Nature* 289: 549-554.
- Zitron I.M. and B.L. Clevinger 1980 Regulation of murine B cells through surface immunoglobulin. I. Monoclonal anti- δ antibody that induces allotype-specific proliferation. *J. Exp. Med.* 152: 1135-1146.
- Zola H. 1980 Monoclonal antibodies against human cell membrane antigens: A review. *Pathology* 12: 539-557.